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CAM-4, A Portable Warning Device for Organophosphate Hazardous Material Spills

Midwest Research Inst.
Kansas City, MO

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16. ABSTRACT

CAM-4 is a completely portable, battery-operated, field version of the earlier cholinesterase antagonist monitor, CAM-1, which senses organophosphates and carbamates in water supplies. The present report describes the design, fabrication, and evaluation of the CAM-4 device. Like CAM-1, this device uses immobilized cholinesterase in an electrochemical cell for the detection of cholinesterase inhibitors in water supplies. CAM-4, however, is not fully automated and therefore requires an operator to observe the cell voltages recorded by the digital printer and to decide whether toxic levels of pesticides have been sampled. The elimination of automation provided in CAM-1 was a trade-off to provide 8-hr operation from a standard size 12-v automobile battery and to keep the weight and cost of the instrument low. Sub-toxic levels of Mesurol^{R}, Nemacur^{R}, Baytex^{R}, DDVP, Systox^{R}, Furadan^{R}, and Sevin^{R} were all detected by CAM-4 at sensitivities comparable to those obtained with CAM-1 (see Task 1 report on this contract, EPA-600/2-77-219, November 1977).

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FOREWORD

When energy and material resources are extracted, processed, converted, and used, the related pollutional impacts on our environment and even on our health often require that new and increasingly more efficient pollution control methods be used. The Industrial Environmental Research Laboratory - Cincinnati (IERL-Ci) assists in developing and demonstrating new and improved methodologies that will meet these needs both efficiently and economically.

This report describes the design, fabrication, and preliminary evaluation of CAM-4, a portable version of the earlier Cholinesterase Antagonist Monitor, CAM-1. Both instruments use immobilized cholinesterase in an electrochemical cell in order to sense the presence of organophosphates and carbamates in water. CAM-4 operates equally well from a 110-v AC line at a fixed facility or from a 12-v battery. Battery-powered units have been used repeatedly on land and from boats. CAM-4 is useful for locating pesticide discharges into streams and also for following the movement of pesticide spills in lakes and streams. Information on this subject beyond that supplied here may be obtained by contacting the Oil and Hazardous Materials Spills Branch, IERL-Ci, U.S. EPA, Edison, NJ 08817.

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ABSTRACT

Previously, an instrument designated as CAM-1 ("CAM" is an acronym for "cholinesterase antagonist monitor") was constructed to continuously monitor the levels of organophosphate and carbamate pesticides in water (ponds, streams, plant outfalls, etc.). CAM-1 is a sophisticated research instrument that cannot be conveniently used in the field or from a boat because of its non-ruggedized construction. To meet the requirements for an equally sensitive but portable system, CAM-4 was developed. CAM-4 is a field version of CAM-1 that will operate continuously in the field from a 12-v DC power supply for eight hours, or from 110-v AC. The present report describes the design, fabrication, and evaluation of CAM-4.

Operation of the CAM instruments is based on inactivation of the enzyme cholinesterase by organophosphate and carbamate pesticides. The extent of inactivation, which is proportional to the amount of inhibitor present, is determined by measuring the response of the system to a substrate readily hydrolyzed by the enzyme.

The conventional method for detecting organophosphates and carbamates in water requires the addition of cholinesterase, a buffer, and an enzyme-hydrolyzable substrate, e.g., butyrylthiocholine iodide, to a water sample, followed by spectrophotometric determination of the residual substrate concentration. The spectrophotometric method is disadvantageous because: (a) a significant quantity of costly enzyme is needed for each test, and (b) the system is not easily adapted to continuous use since the optical windows become dirtied by the flow of contaminated water. It is necessary to correct this problem by comparing the response of the contaminated sample, plus reagents, to that obtained with a contaminated sample alone. However, even with this correction, frequent cleaning is necessary. The CAM systems eliminate these problems. Entrapping a given amount of enzyme in starch gel on the surface of open-pore polyurethane foam makes repeated use possible over extended periods of time. Residual activity of the enzyme is measured electrochemically.

In the CAM system, residual activity is determined during a sampling cycle by the level of substrate hydrolysis product present in the electrochemical cell. Presence of a cholinesterase inhibitor reduces the rate of substrate hydrolysis (thiol formation) and produces an increase in cell voltage. The magnitude of the increase above the enzyme pad potential is a function of the residual enzyme activity; that is, the voltage rises as the enzyme is inhibited. A voltage increase from one sampling cycle to the next, above a designated alarm threshold, is used to trigger an "alarm" indicating the presence of cholinesterase inhibitors.

Development of a portable system necessitated elimination of the following convenience features from CAM-1: the computer logic circuits, automatic pad changer, digital voltmeter, strip chart recorder, and audible alarm components. CAM-4 contains an inverter to transform DC to AC current and a digital printer to record cell voltage. An operator must read the digital printout to determine when there is an "alarm" condition and when it is necessary to replace the enzyme pad.

CAM-4 possesses detection and monitoring capabilities equal to those of CAM-1. The sensitivity of CAM-4 to subtoxic levels of DDVP, Systox^{R}, Furadan^{R}, malathion, Sevin^{R}, and other pesticides in water is comparable to the sensitivity of CAM-1 for the same pesticide solutions.

This report was submitted in fulfillment of Contract No. 68-03-0299, Task No. 2, by Midwest Research Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from April 3, 1975 to May 3, 1976, and work was completed as of July 3, 1976.

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ACKNOWLEDGMENTS

The work upon which this publication is based was performed pursuant to Contracts Nos. 68-01-0038 and 68-03-0299 with the Environmental Protection Agency. This report describes the work done on Task II of the latter contract. Task I was concerned with the evaluation of the Cholinesterase Antagonist Monitor Model No. 1 (CAM-1) with a series of commercially available pesticides including both organophosphates and carbamates. Task II is concerned with the design, fabrication, and evaluation of a portable version of CAM-1, which has been designated as CAM-4. Task III is concerned with the investigation of alternate enzyme systems for use in CAM-1 and CAM-4; this work has been partially funded and is now in progress.

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SECTION 1

INTRODUCTION

Spills or discharges of hazardous materials into rivers, streams, or lakes create the danger that toxic levels of chemicals could result in a massive fish kill or that the polluted water could enter a municipal water supply. Hazardous material spills must be detected promptly and followed so as to minimize health and environmental effects.

In an earlier report (EPA/R2-72-010), the Cholinesterase Antagonist Monitor (CAM-1), which utilized cholinesterase immobilized on a polyurethane pad in an electrochemical cell for detecting and/or monitoring cholinesterase antagonists in water, was described. This instrument provided a response on a real-time basis to organophosphate and carbamate pesticides in water. An abstract of Report EPA/R2-72-010 is included as Appendix A.

Task I on the present contract reported the sensitivity of CAM-1 to a series of pesticides and was approved for publication in August 1977 (EPA-600/2-77-219). Such a study was necessary since the sensitivity of the instrument was directly related to the affinity of cholinesterase for the pesticide being detected. In most cases, the sensitivity of the instrument was also related to the toxicity of the material being detected, since the mechanism of toxicity and the mechanism of detection are both concerned with the binding of the toxic material to cholinesterase. The abstract of EPA-600/2-77-219 is included as Appendix B.

CAM-1 was designed for use at fixed locations where power was readily available and where mobility was not a major consideration. One such application that had been envisioned was its use at the intake of a municipal water supply. Here, the instrument would detect pesticide spills occurring upstream from the water inlet upon their arrival at the inlet pipe and provide a warning so that the inlet valve could be shut until the spill had passed.

The objective of Task II on this contract has been the construction and preliminary evaluation of a portable version of CAM-1 to which we have assigned a code designation CAM-4. It was planned that this instrument could be operable from a boat using a 12-v DC power supply and that it could be taken as needed to monitor the movement of spills and the effectiveness of clean-up procedures. The present report describes the construction and operation of CAM-4, the portable version of CAM-1.

In the CAM systems, the immobilized horse serum cholinesterase serves as a dosimeter in that it collects pesticides (irreversible cholinesterase inhibitors) on its active sites with a resulting decrease in enzyme activity.

The enzyme itself is entrapped on open-pore polyurethane foam pads with aluminum hydroxide and starch gel; this permits its use for extended periods of time when inhibitors are absent from the water sampled.

In addition to being a pesticide collector, the immobilized enzyme also serves as a biological amplifier for the measurement of residual enzyme activity following its exposure to an unknown water sample; thereby, it enables the CAM instruments to determine the presence or absence of cholinesterase inhibitors in the water sampled.

Although either spectrophotometric or electrochemical methods could have been used to monitor the rate of formation of substrate hydrolysis products by the immobilized enzyme pad, we chose to use the electrochemical method since it was cheaper to fabricate and less subject to problems caused by dirt and air bubbles in the water samples. In the CAM systems, the substrate, i.e., butyrylthiocholine iodide, is hydrolyzed by the enzyme to give the easily oxidizable substance, thiocholine iodide. This latter compound is then detected by the application of a constant current ($\sim$ 2 μA) to platinum electrodes in contact with the enzyme pad; thus, when enzyme inhibitors are absent, the enzyme is active and there is an abundance of thiocholine iodide present, which causes a low potential ($\sim$ 200 mv) between the two electrodes. On the other hand, when enzyme inhibitors are present, the activity of the enzyme is low, the quantity of thiocholine iodide formed is small, and the potential between the electrodes is $\sim$ 500 mv. Thus, it is possible to tell whether the sampled water contained enzyme inhibitors by observing the potential between the electrodes.

The reason for the voltage change in the CAM electrochemical cell is not entirely understood. One hypothesis suggests that the low voltage between the electrodes when enzymes are present is due to the simple electrooxidation of the thiocholine iodide to the corresponding disulfide. The other explanation says that the anode is coated with a layer of platinum oxides or sulfides and that exposure of this coating to the thiol compounds results in a partial depolarization of the anode. The arguments for and against the two theories explaining the generation of characteristic voltages are given in Appendix E (page 53) and in the Task I report on this contract (EPA-600/2-77-219). In the latter report may be found detailed discussions of the operating principle of the electrochemical enzyme cell, the studies related to the selection of buffer, pH and concentration, the effect of temperature on the operation of the system, the response of CAM-1 to many additional organophosphate and carbamate pesticides, the response to reversible inhibitors, the response to possible interfering substances, a procedure for fabricating the enzyme pads for the electrochemical cell, and other subjects related to operation of CAM-type monitors.

The operating principles for CAM-1 and CAM-4 are identical even though there are some differences in the degree of automation, the specific operating procedures, and the power requirements. The following sections of this report describe the construction, operation, and preliminary evaluation of the portable, battery-operated pesticide monitor known as CAM-4.

SECTION 2

CONCLUSIONS

On the basis of the present investigation of immobilized enzymes for the detection of toxic organophosphate and carbamate insecticides in water, the following conclusions have been made:

1. A portable enzyme-based detection system to monitor water supplies for the presence of organophosphate and carbamate insecticides has been designed, fabricated, and tested. The new portable system has been designated as the Cholinesterase Antagonist Monitor Model No. 4, CAM-4. This unit is designed specifically for field use and, like CAM-1, responds rapidly to low levels of cholinesterase antagonists in water supplies, streams, and impoundments.

2. The complete CAM-4 detection system and several of its component parts, including specifically the enzyme cell for water monitoring, have been redesigned to reduce weight and power requirements and to permit easy replacement of the electrochemical cell if this should become necessary.

3. CAM-4 responds to toxic and subtoxic levels of organophosphate and carbamate insecticides (the levels referred to are based on rat and animal toxicity data). In a response test series with city tap water at 25°C to which 0.2 ppm of DDVP (dimethyl 2,2-dichlorovinyl phosphate) had been added, CAM-4 provided repeated voltage increases equal to or exceeding the 10-mv alarm threshold. On this basis, it is concluded that CAM-4 has adequate sensitivity to provide adequate warning of situations that may lead to accidental poisoning of human and other animal species by cholinesterase inhibitors in water supplies.

4. Like CAM-1, CAM-4 responds to toxic and subtoxic levels of many organophosphate and carbamate insecticides, including the following that have been tested: Baytex^{R}, Sevin^{R}, Mesurol^{R}, Guthion^{R}, malathion, Furadan^{R}, Baygon^{R}, Systox^{R}, and Nemacur^{R}.

5. The sensitivity of CAM-4 to low levels of insecticides in water is the result of the affinity of these insecticides for the reactive sites on the surfaces of the immobilized enzyme used in the electrochemical enzyme cell. Thus, the enzyme is functioning as a selective concentrator (or dosimeter) for the materials to be detected.

6. CAM-4 is the first portable detection instrument that has successfully used an immobilized enzyme product for the automatic monitoring of water supplies for the presence of enzyme inhibitors.

7. CAM-4 operates satisfactorily with enzyme pads fabricated from open-pore urethane foam, starch gel, and cholinesterase complexed with aluminum hydroxide gel.

8. The present CAM-4 operates on a 3-min detection cycle in which water is sampled for 2 min and the pad activity (measured electrochemically) is determined during the third minute. The sensitivity of the system can be increased by manually increasing the water sampling period to 6 min using the controls provided.

9. The CAM-1 developed earlier is recommended for fixed installations and is suggested for use by operators of water treatment facilities to warn of pesticides in water supplies; CAM-4, however, is portable and is recommended for detecting or following the movement of spills in lakes or streams so that appropriate action may be taken at the spill or discharge site.

10. CAM-4's ability to detect insecticides in water is based upon a biochemical reaction known to be involved in animal toxicity.

11. CAM-4 is not fully automated, but it is portable and can be operated by one person in a small boat or at any location where either 12-v DC or 110-v AC power is available.

12. CAM-4 will operate continuously for at least 8 hrs from a 12-v automobile battery (55 amp-hr capacity).

13. Unlike CAM-1, CAM-4 requires an operator in attendance during its use in water monitoring. The digital printer on CAM-4 provides the operator with information about the condition of the enzyme pad and the presence or absence of inhibitors in the water sampled once each detection cycle. Although the operator must decide when to change enzyme pads and when toxic levels of pesticides are sampled, the omission of some memory and logic circuits--incorporated in CAM-1--has greatly simplified the construction of the instrument and lowered the cost without reducing its sensitivity or response time. It also provides a permanent time-based recording showing when spills are detected.

14. CAM-4 can probably be modified to reduce its power consumption when it is operated on battery power if the power requirements prove to be excessive. It is visualized that power can be saved if the various pumps and circuits are designed to operate on direct current without an inverter. Operation on alternating current could then be accomplished with a 110-v AC to 12-v DC power supply; with this design, the major power loss would occur when CAM-4 is connected to a 110-v AC line.

SECTION 3

RECOMMENDATIONS

1. CAM-4, the portable version of the Cholinesterase Antagonist Monitor, CAM-1, should be tested more extensively in the field to establish its versatility and reliability for monitoring of pesticide spills in lakes and rivers. Such further testing is needed primarily to encourage the use of CAM-4 in the solution of practical problems encountered by governmental agencies and private industries. For example, the sampling of outfalls from manufacturing and formulating plants can be conveniently accomplished by one man in a boat equipped with CAM-4. Another application is to use CAM-4 from a boat to map the extent of a pesticide spill in a lake or to monitor the effectiveness of a cleanup procedure in removing pesticide from a body of water.

2. It is recommended that CAM-4 be used to detect those pesticides detectable by CAM-1 where portability is an advantage.

3. Additional studies of the performance of either CAM-1 or CAM-4 should be conducted to determine the possible adverse effects of various organic and inorganic water pollutants that may occur in industrial or agricultural wastewaters.

4. The response profile of CAM-1 and/or CAM-4 should be further evaluated by an exposure of the detector to a wide variety of pesticides or other potential water pollutants. This study should be concerned not only with the detection of compounds, but also with establishing the thresholds at which these chemicals are detected.

5. Studies should be conducted in which the objectives are: (a) to determine the reliability of the various CAM-4 components, and (b) to improve reliability of the components and whole system when failures or malfunctions occur. Testing of this type is needed to provide the assurance of trouble-free operation under anticipated field conditions and to generate recommended servicing procedures and schedules.

SECTION 4

DESIGN AND FABRICATION OF THE PORTABLE CHOLINESTERASE ANTAGONIST MONITOR (CAM-4)

Before designing or constructing the portable water monitoring system based upon the use of immobilized cholinesterase as the sensor, it was necessary to consider the intended use of the detector and the manipulations and skills required of the operators, as well as the sensitivity, selectivity, permissible size, weight, cost, need for maintenance, and other parameters affecting the design.

From the start, it was planned that the CAM-4 should be as much like CAM-1 as possible, but it should be operable from 110-v AC and 12-v DC power sources. Capability for operation of the instrument from a 12-v DC power source was expected to expand its usefulness in the field since power would be available from cars, trucks, boats, or inexpensive storage batteries. It was required that the instrument in its case be watertight, occupy a space of approximately 1 cu ft (0.03 m^3) and weigh less than 30 lb (14 kg) excluding the battery.

A large share of the cost and complexity of the laboratory model (CAM-1) is directly related to components that have been eliminated from the field model (CAM-4). The items eliminated include: the automatic enzyme pad changer, the strip chart recorder, the digital voltmeter, the horn, and the computer logic circuits. Since there will always be an operator in attendance with the CAM-4, he will decide when to change the enzyme pads or when to signal an alarm. The digital printer is set to print the cell voltage at the end of each 3-min detection cycle; also the printer provides a permanent record of the exact voltages obtained during instrument operation, thus eliminating the need for either a strip chart recorder or a digital voltmeter.

The sensitivity of CAM-4 was expected to be essentially the same as the laboratory model (CAM-1) since the enzyme pads, substrate, electrodes, and timing cycle would be the same in both instruments. As far as possible, the individual operating procedures were to be as simple as possible so that a minimum of effort or thought would be required from the operator in order to obtain reliable data.

The portable Cholinesterase Antagonist Monitor (CAM-4), which has been designed and fabricated for rapid detection of organophosphates and carbamates in water supplies, is shown in its fiberglass carrying case in Figure 1.

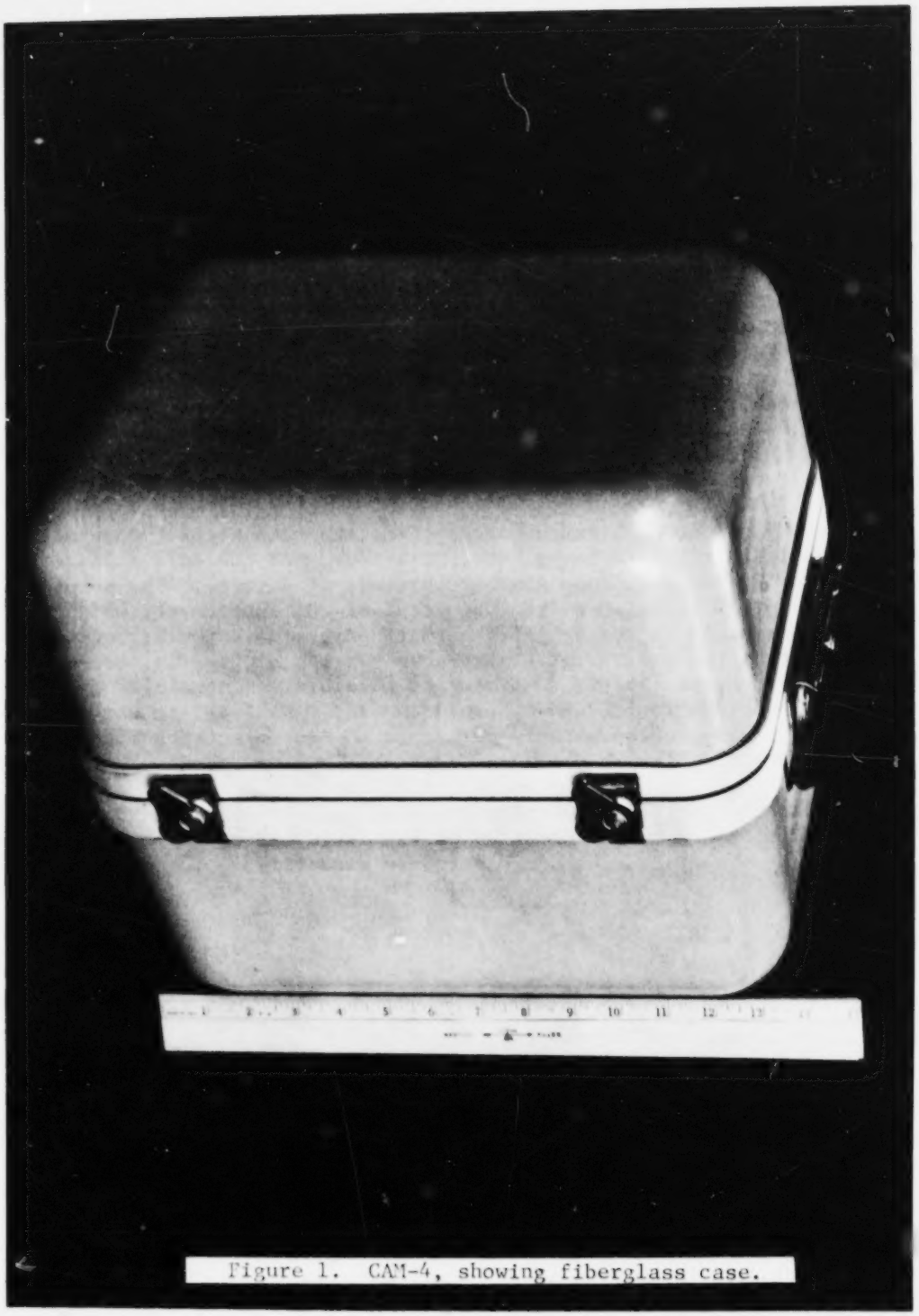


Figure 1. CAM-4, showing fiberglass case.

The instrument is designed so that most of the electrical components are located in the right half of the case while the mechanical components (pumps, motors, cell holder, etc.) are located in the left half of the case. Figure 2 shows the location of these components.

In the upper right corner are the manual control switches for water, substrate, and current. Each has a red indicator light above it that comes on in sequence and tells which part of the detection cycle is in progress. Any part of the cycle may be extended by manually flipping the proper toggle switch up, but all of these switches must be in the "down" position for the CAM-4 detection to operate automatically.

To the left of the manual controls on the front panel is the digital printer; it has a manual print-paper advance switch, a low-paper indicator, and a thumbscrew for removal of the assembly during paper reloading. In the middle of the panel are located the fan for cooling the electronics, the AC-DC on-off switch, and separate fuses for AC and DC operation. The 10 A fuse is used when operating from an AC power source. At the bottom of the panel are located the AC input plug, the DC input plugs, and the power inverter for generating 110-v AC during battery operations.

The left panel of the instrument contains the mechanical components. These include the cell open lever, the pad holder, and the cell voltage jack from which an external recorder may be operated, if desired. The substrate pump is immediately accessible from the front of the instrument, while the water pump is reached by removal of the small pump access panel, located at the lower left of the instrument. Also shown in the picture is the location of the substrate supply bottle. Insofar as possible, commercially available parts for the fabrication of CAM-4 have been used. However, it was necessary to design and fabricate the manual enzyme pad changer-electrochemical cell assembly, a view of which is shown in Figure 3.

The detection cycle is presented graphically in Figure 4. The following description of the automatic operation of the detection cycle in CAM-4 shows the time sequence activated by the electronic circuitry.

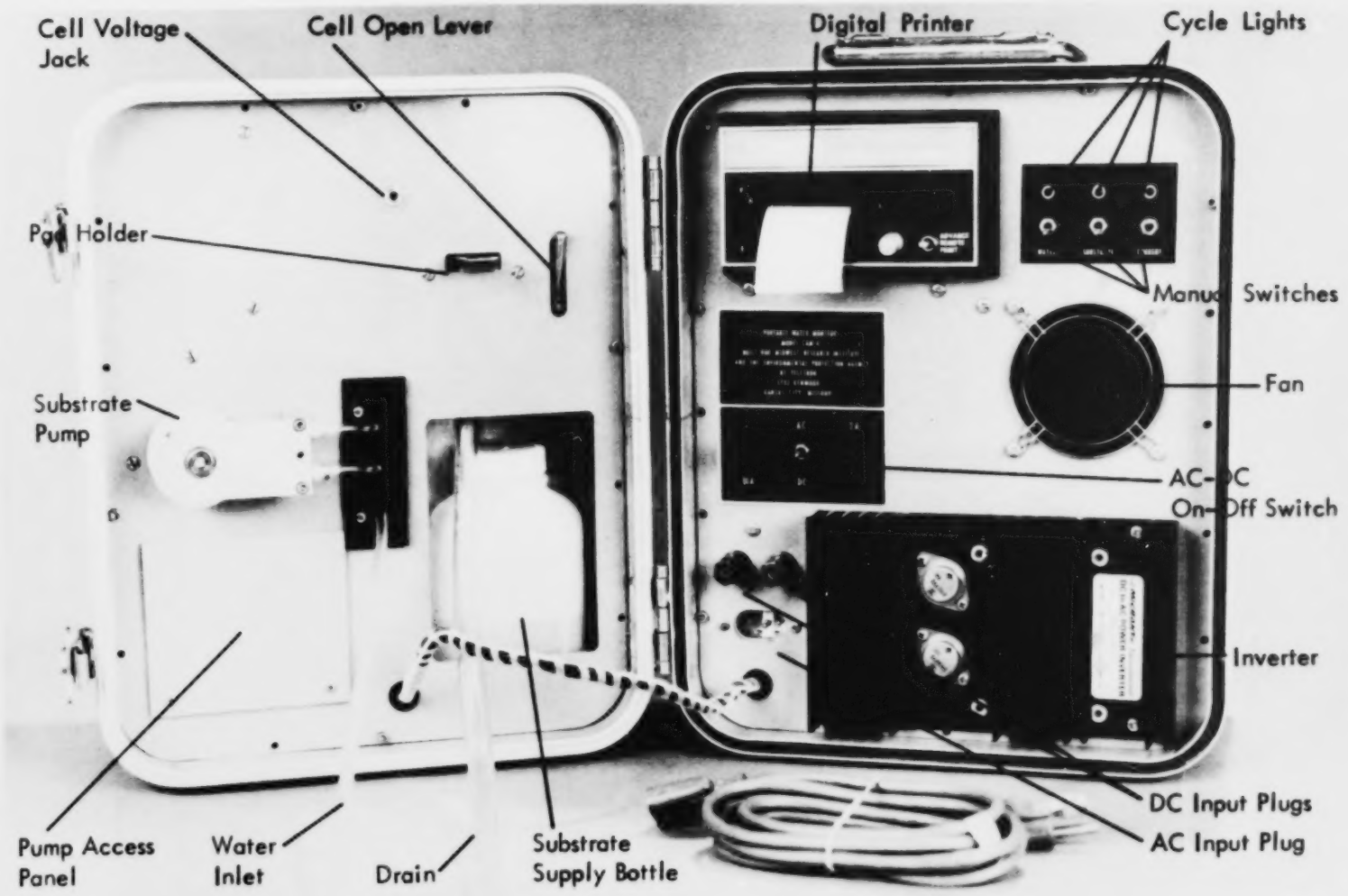


Figure 2. Internal view of portable Cholinesterase Antagonist Monitor, CAM-4.

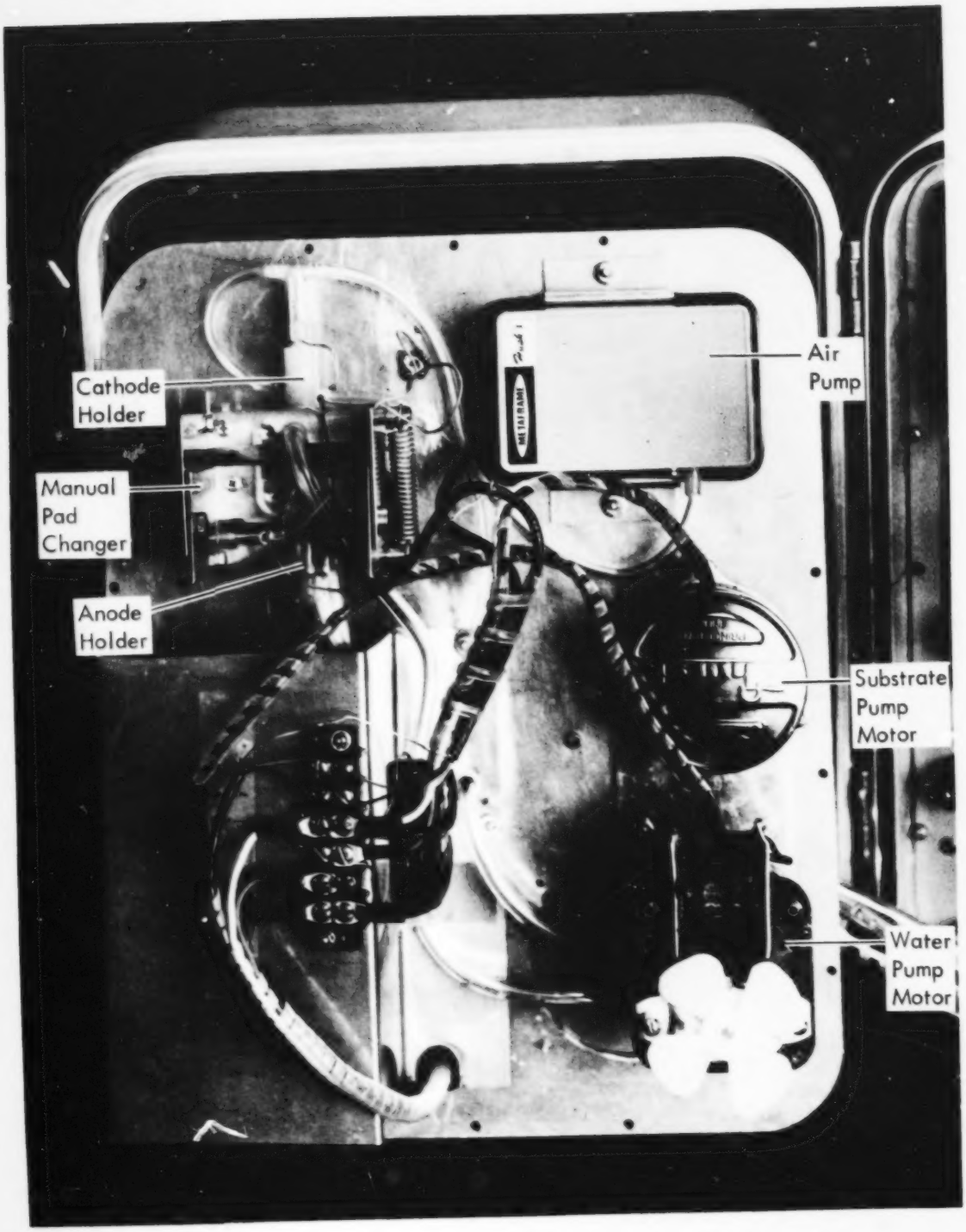


Figure 3. View of manual enzyme pad changer and motors in CAM-4 (behind left panel).

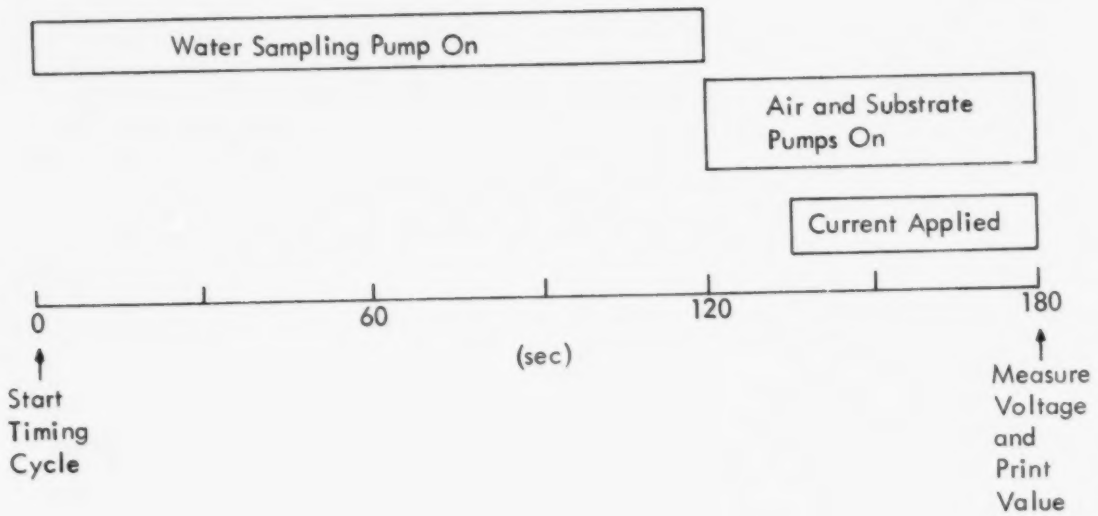


Figure 4. CAM-4 3-min operating cycle for collecting enzyme inhibitors on an immobilized enzyme pad and subsequently measuring a voltage related to the enzyme activity of the pad.

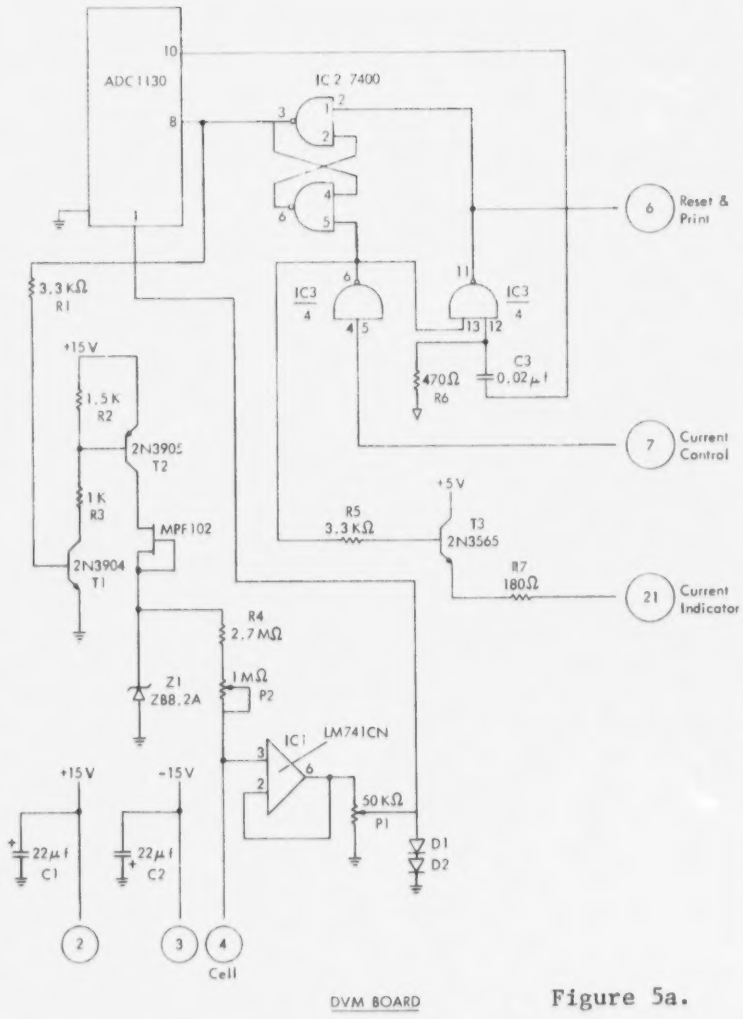
0 sec	Everything off.
1 sec	Turn water pump on.
120 sec	Turn water off; start air and substrate flow.
140 sec	Apply constant current to electrochemical cell.
180 sec	Print voltage on digital printer and advance paper one line. Turn off substrate solution and applied current and reset clock to "0".

As mentioned earlier, the decision to incorporate a 12-v DC to 110-v AC inverter was based upon several considerations. Principally, the water, air, and substrate pumps and the digital printer all require 110-v AC or DC voltages differing from 12 v. Although DC motors might have been obtained for these applications and the printer modified to operate on 12 v, it was easier to obtain and service commercially available 110-v equipment than 12-v equipment. A further consideration involved the need to operate the substrate pump at constant speed to assure a flat baseline voltage. Constant speed DC pump motors in the appropriate size are more expensive and more difficult to control than are commercial AC motors.

Figures 5a, 5b, 6, 7, and 8 are the wiring diagrams for the CAM-4 system. Figures 5a and 5b show the "DVM" board, which provides the analog-to-digital conversion for the signal obtained from the cell voltage amplifier. Also shown in these drawings are the constant current source for the electrochemical cell and the regulated power supplies for the remainder of the circuits.

Figure 6 presents the wiring of the 12-v DC to 110-v AC inverter and also the switching gear for operation of CAM-4 from either AC or DC power. Figure 7 depicts the circuitry for the time circuit and the optically coupled triac switching circuits for the pumps. Figure 8 shows the interconnections of the panel switches, the digital printer, the printed circuit boards, and the other components.

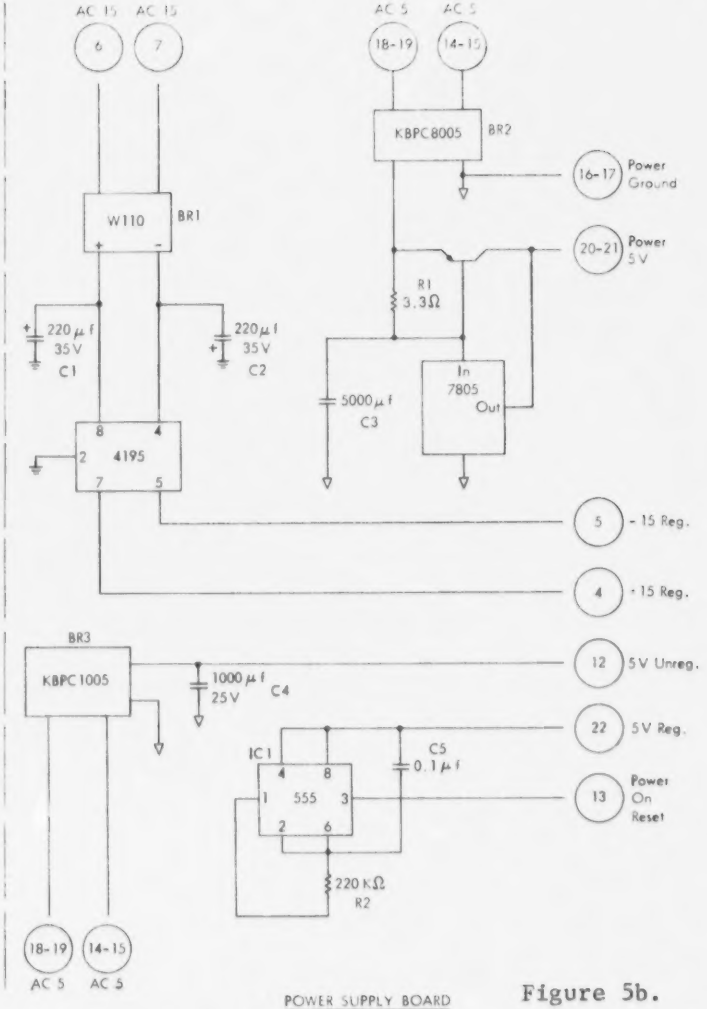
The electrochemical cell design adapted for use in the CAM-4 is presented in Figure 9. The immobilized enzyme pad is placed between the two perforated platinum electrodes, which are held in the injection molded holders made of Cyclolac plastic. These electrode holders are held under constant spring tension against the enzyme pad holder to form a leak proof seal--the O-ring between each surface is not shown in the drawing. The constant current of $\sim$ 2 μA applied to the electrodes once each cycle is supplied by a regulated 8.5-v DC power supply with a 2.7-megohm resistor and a 1-megohm potentiometer in series.



DVM BOARD

Figure 5a.

Analog to digital converter and cell signal amplifier and current source.



POWER SUPPLY BOARD

Figure 5b.

Power supply board providing regulated +5 and ±15-v DC for logic boards and power for printer.

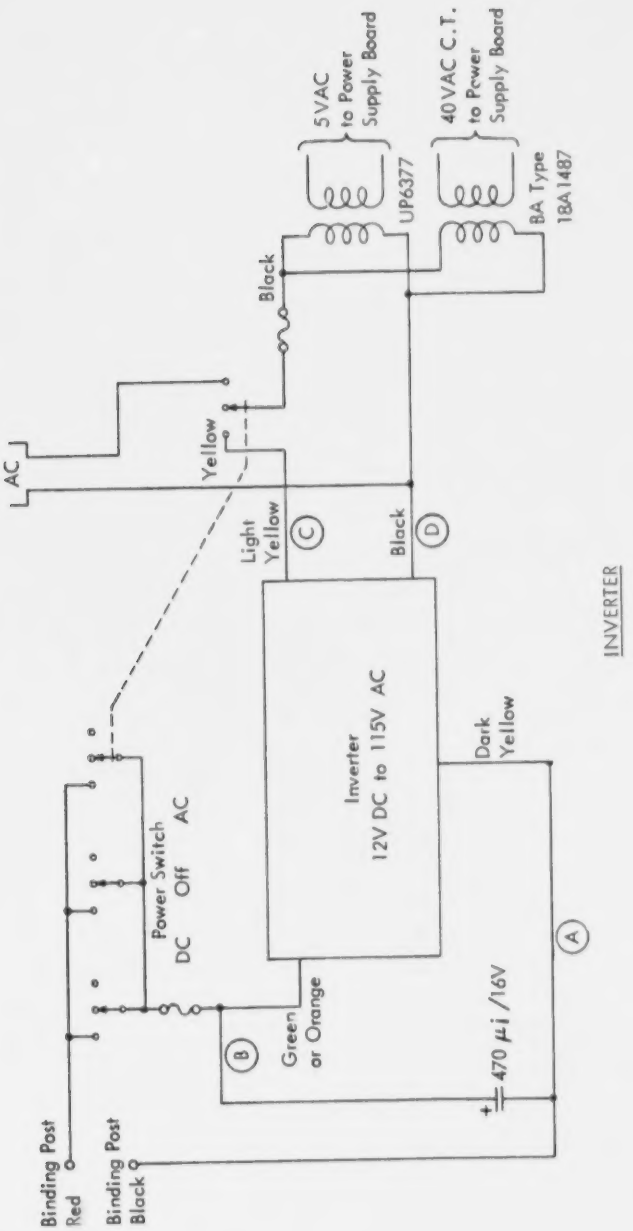


Figure 6. Wiring diagram showing 12-v DC to 110-v AC inverter and DC/AC switching.

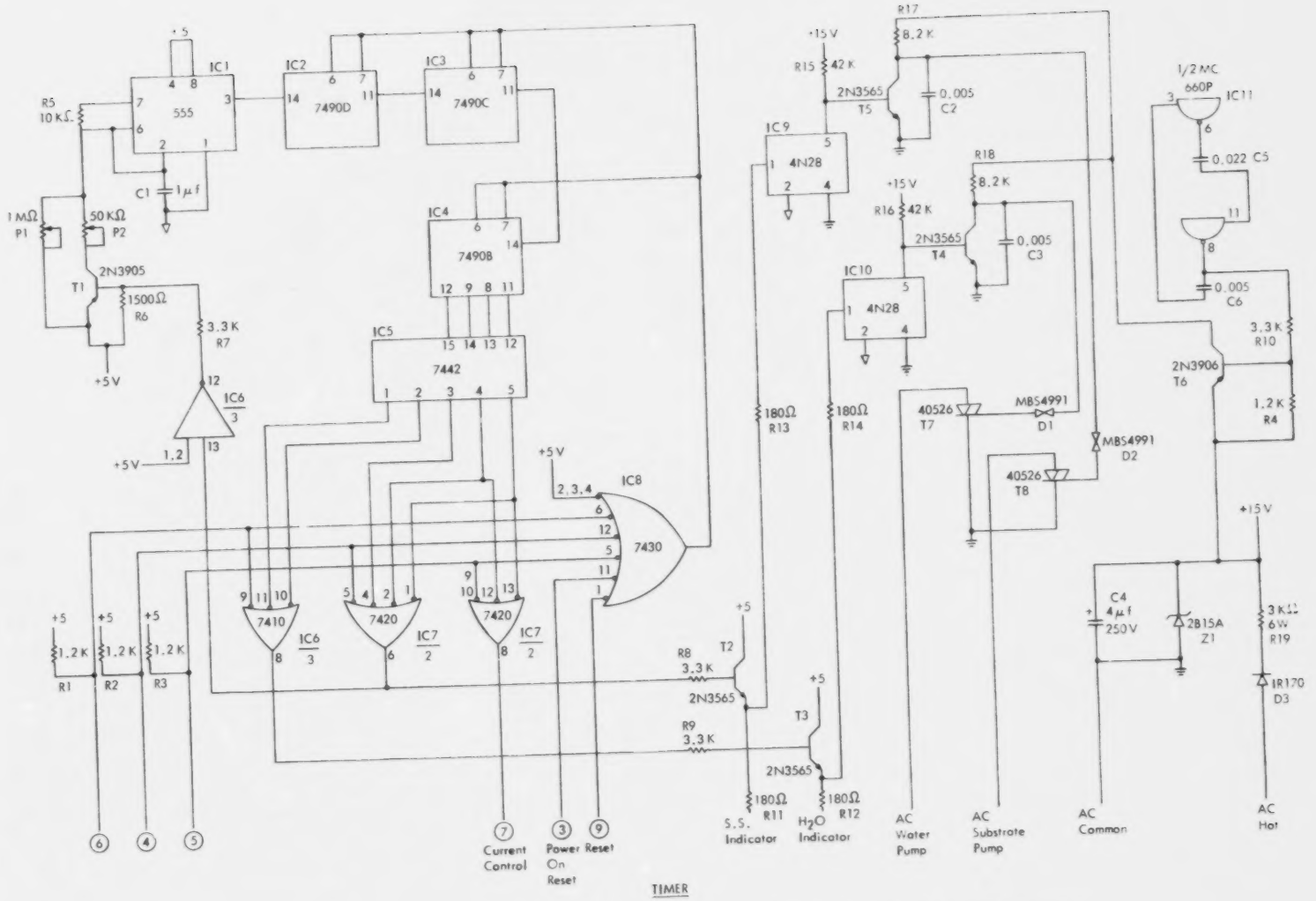


Figure 7. Timer and optically coupled triac switching circuits for pumps.

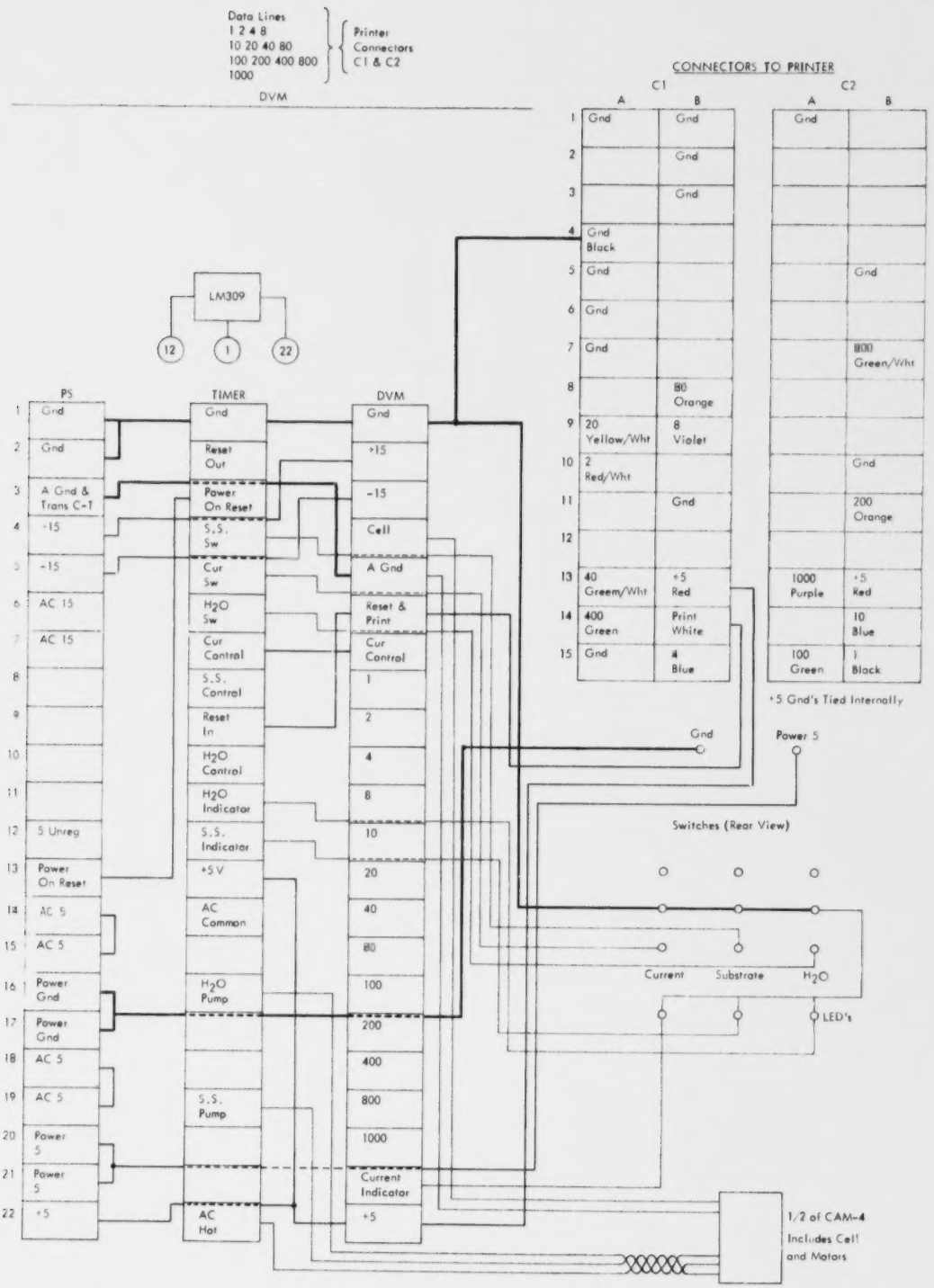


Figure 8. Wiring diagram showing interconnection of panel switches, digital printer, printed circuit boards, and other components.

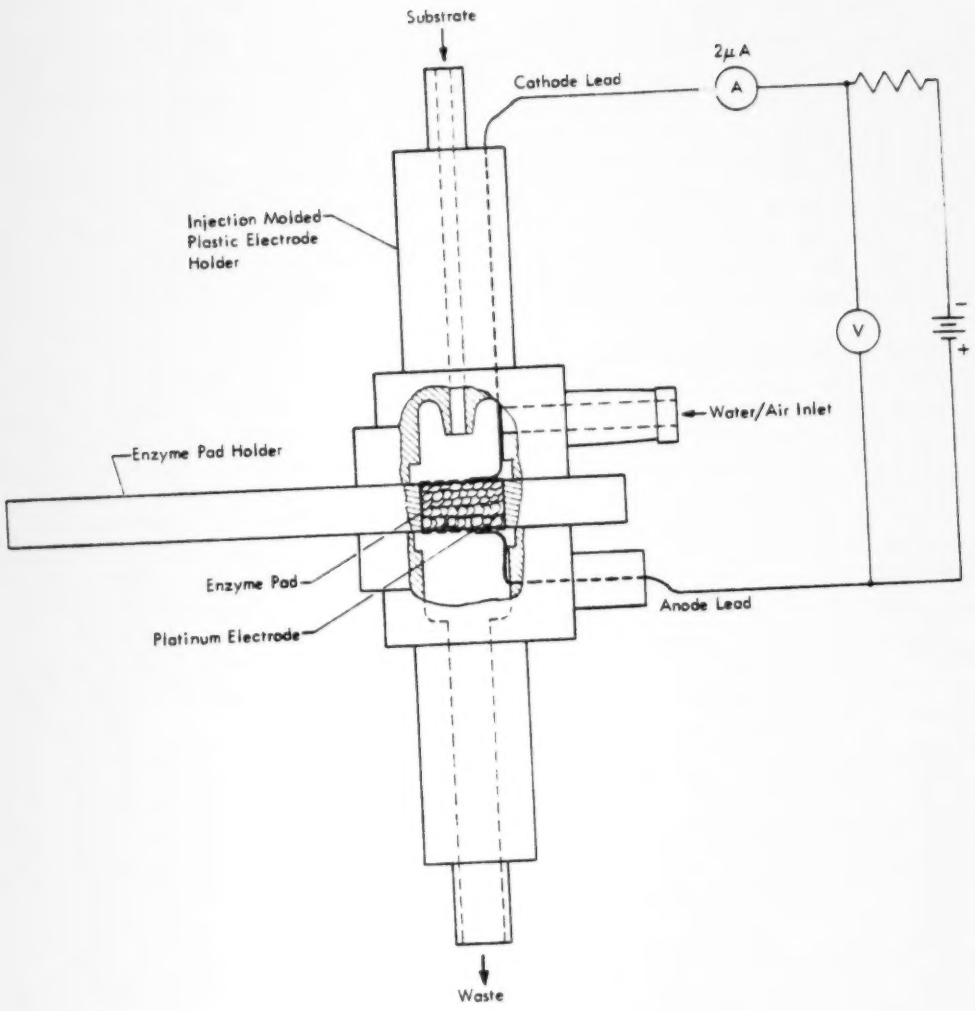


Figure 9. Cross section of an electrochemical cell developed for water monitoring showing the platinum electrodes above and below the enzyme pad to which a constant current is applied.

A brief description of the operation of the electrochemical cell for the generation of electrochemical voltages related to the activity of the enzyme pad between the electrodes follows:

During the 2-min water sampling portion of the 3-min detection cycle, the water is pumped through the immobilized enzyme pad at ∿ 200 ml/min.* Enzyme inhibitors, if present, are collected on the enzyme pad, where they inhibit all or part of the active sites on the enzyme pad. During the enzyme testing portion of the detection cycle, air at ∿ 2 liters/min is pumped through the cell continuously to remove the excess liquid from the enzyme pad. At the same time, a solution of butyrylthiocholine iodide (BuSChI) is pumped through the enzyme pad; during the last 40 sec of the enzyme testing part of the cycle, a 2-μA constant current is applied and the resulting cell voltage is recorded by the digital printer. (See Figure 4 for the complete detection cycle.)

When this system is functioning normally in the absence of inhibitors, the substrate is cleaved by the enzyme to produce a thiol, which contacts the lower platinum electrode (the anode). In this case (no inhibitors present), a voltage of approximately 200 mv is produced, which is characteristic of a new working enzyme pad. In the case where the enzyme is completely inhibited (or when a blank pad is isolated in the electrochemical cell), voltages of 400 mv or more are obtained. Intermediate voltages are generated in proportion to the extent of inhibition of the enzyme. In actual operation of the system, a voltage rise of 10 mv or more per cycle is considered to be an "alarm" condition; a single enzyme pad may signal ten or more alarms before it must be replaced with a fresh enzyme pad. However, exposure of an enzyme pad to high levels of inhibitor could inhibit all of the enzyme in one or two cycles.

* This pumping rate is less than half of the water flow rate in CAM-1. However, there is little loss in sensitivity with the lower pumping rate since the diffusion of the inhibitor to the active sites on the enzyme is the rate limiting reaction.

SECTION 5

CAM-4 OPERATING PRINCIPLE

The operating principle for CAM-4 is as follows: an electrochemical process is used for the automatic determination of the activity of the enzyme pad once during each 3-min cycle. As may be seen from the cross section of the electrochemical enzyme cell shown in Figure 9, the porous enzyme pad is located between two porous platinum electrodes. First, the water sample is pumped through the enzyme pad, at approximately 200 ml/min, for 2 min during which time a portion of the enzyme inhibitor in the water combines with the active sites on the enzyme to reduce the enzyme activity. At the end of the 2-min water sampling period the water is turned off and air is blown through the enzyme pad to displace any residual water. Next, a solution of substrate, consisting of butyrylthiocholine iodide in Tris buffer, is pumped through the enzyme pad at the rate of 1 ml/min for a period of 1 min; during the last two-thirds of this substrate pumping cycle a constant current of 2 μA is applied to the platinum electrodes so that the lower electrode is positive (anode) and the upper electrode is negative (cathode). In the absence of enzyme inhibitors, the cholinesterase (ChE) hydrolyzes the substrate to thiocholine iodide, which possesses a thiol group that produces characteristic low voltages in the electrochemical cell.

$$C_3H_7COSCH_2CH_2\overset{+}{N}(CH_3)_3I^- \xrightarrow{ChE} C_3H_7COOH + HSCH_2CH_2\overset{+}{N}(CH_3)_3I^-$$

$$\downarrow \text{electrochemical oxidation}$$

$$(CH_3)_3\overset{+}{N}-CH_2CH_2-S-S-CH_2CH_2\overset{+}{N}(CH_3)_3 2I^-$$

On the other hand, in the presence of enzyme inhibitors, the substrate is not hydrolyzed; there is no thiol formation and the cell voltage rises ∿ 200 mv (from ∿ 200 mv to ∿ 400 mv). Thus, a low cell voltage is indicative of the absence of enzyme inhibitors, while an increase in cell voltage means that all or part of the enzyme activity has been removed by an inhibitor present in the sampled water.

The cell voltage changes observed in the operation of CAM-4 resemble those observed for CAM-1. Consider CAM-1 response to water containing 0.2 ppm of DDVP (dimethyl dichlorovinyl phosphate). In this case, the voltage tracing shown in Figure 10 was generated by applying a constant current to the enzyme pad during each cycle. Upon encountering a cholinesterase inhibitor, a change in voltage is recorded. The voltage change from cycle to cycle is used to trigger an alarm. As shown in Figure 10, during the first 24 min there is a very slow steady voltage rise from cycle to cycle, indicating the gradual deterioration of the immobilized enzyme pad. The alarm

level is set so that these changes are too small to trigger an alarm. However, when 0.2 ppm of DDVP is added to tap water, a sharp increase in the height of the voltage peak occurs. Each cell voltage increase of 10 mv or more between cycles is considered to be an alarm. (The 10-mv alarm threshold can be changed, if desired.) In the present example, 10 individual alarms from the same enzyme pad resulted from sampling the DDVP. If a higher concentration of DDVP--perhaps 2 ppm--had been used, the cycle-to-cycle voltage increases would have been much greater.

Enzyme pads cannot be used indefinitely. After a time, when there is insufficient enzyme activity on the enzyme pad to allow a 50-mv voltage rise when inhibitors are sampled, the used enzyme pad must be rejected as a safety factor and a new pad inserted into the system by the operator.

Like CAM-1, the portable water monitor produces voltage increases when cholinesterase inhibitors are present in the water. Unlike CAM-1, however, the portable CAM-4 does not trigger an alarm, but relies on the operator to determine when inhibitors are present and when to replace enzyme pads.

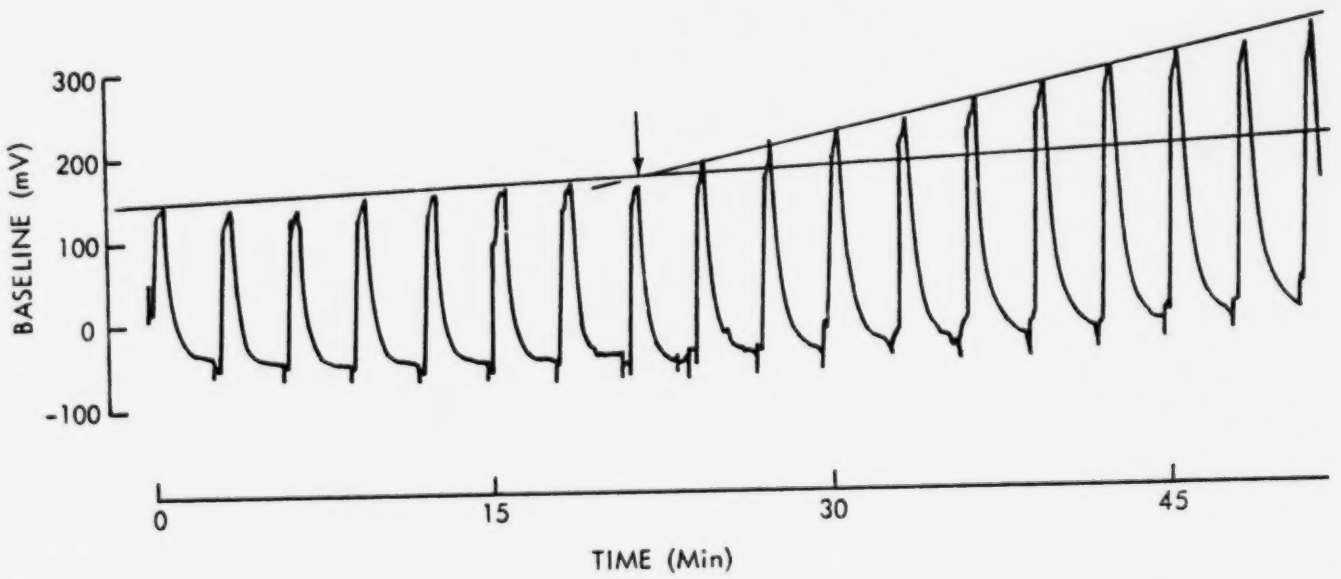


Figure 10. Response of the electrochemical cell operating on the 3-min cycle to water containing 0.2 ppm DDVP.

SECTION 6

OPERATING PARAMETERS FOR THE ELECTROCHEMICAL ENZYME SENSOR

An explanation has been given of the basic principles involved in the operation of an electrochemical cell for the detection of low levels of organophosphates in water supplies (Sections 1 and 4, and Appendix E). In order to make this electrochemical enzyme cell both sensitive and reliable for the detection of enzyme inhibitors, it was necessary to control those variables that affected the response of the cell: (a) the buffer solution; (b) the substrate; (c) the applied current; (d) the rate of water sampling; (e) the time of water sampling, and the like. Some of these variables were interdependent; others were not.

Tris buffer {tris(hydroxymethyl)aminomethane-hydrogen chloride} was selected for use in our system because it was compatible with the platinum electrodes, the enzyme, and the substrate. The selection of the buffer concentration at 0.08 M was arbitrary, and could have been changed to 0.10 or 0.15 M with little effect on the response of the unit to enzyme inhibitors. However, the buffering capacity of the 0.08 M substrate is adequate for this system. The pH of 7.4 for the buffer is a compromise between a mildly acidic pH where the substrate is very stable and pH 8.6 where the cholinesterase was especially active in hydrolyzing the substrate, butyrylthiocholine iodide (BuSChI). At pH 7.4, the enzyme is quite active and the spontaneous hydrolysis of the substrate is slow enough at room temperature so that it usually is not a problem. That is, a substrate solution prepared for use in the CAM-4 is still usable after 12 hr at 25°C. A procedure for making the enzyme pads used in the electrochemical cell is given in Appendix F.

Although much information regarding the theory of operation of CAM-type pesticide monitors is given in the present report, the reader is urged to consult the formal report on Task I of the present contract (EPA-600/2-77-219, November 1977) for details relating to CAM-1 performance. In that report may be found detailed discussions of the operating principle of the electrochemical enzyme cell, the studies related to the selection of buffer, pH and concentration, the effect of temperature on the operation of the system, the response of CAM-1 to many additional organophosphate and carbamate pesticides, the response to reversible inhibitors, the response to possible interfering substances, a procedure for fabricating the enzyme pads for the electrochemical cell, and other subjects related to operation of CAM-type monitors.

The following CAM-4 operating parameters have been selected to provide good sensitivity, reliability, and rapid voltage responses when the enzyme is inhibited:

Substrate:	2.5×10^{-4} M butyrylthiocholine iodide in 0.08 M tris(hydroxymethyl)aminomethane-HCl buffer, pH 7.4
Substrate Flow Rate:	1 ml substrate/minute
Enzyme Pad:	0.4-0.8 units of horse serum cholinesterase--entrapped in aluminum hydroxide and starch gels--per enzyme pad
Applied Current:	2.0 μA
Water Sampling Rate:	∿ 200 ml/min

Optimization of substrate concentration and flow rate is crucial since the quantity of substrate reaching the enzyme during each cycle affects the alarm potential, the baseline voltage, and the sensitivity of the detection system.

To maximize sensitivity, on one hand, addition of excess substrate is undesirable, as illustrated by the following example. During each sampling cycle under normal operation, water, contaminated by low levels of inhibitor, flows through an electrochemical cell containing either a fresh or partially inactivated enzyme pad. Then, substrate, in excess amounts, is passed through the cell, as a constant current is applied. Because of the ready availability of substrate, a small amount of enzyme inhibition will have little effect on the rate and extent of hydrolysis (thiol formation). The cell voltage remains low, a characteristic of the absence of enzyme inhibition, in proportion to the amount of enzyme activity. Since an increase in voltage from one cycle to the next is required to signal an "alarm," the presence of the inhibitor is masked.

On the other hand, the use of very small amounts of substrate is also undesirable, as illustrated by the following example. In the case in which low substrate levels are used, very little thiol is formed per cycle and the cell voltage rises, independent of the presence of inhibitors. This voltage increase is seen as a "false alarm." To avoid a continuous "alarm" situation, the alarm threshold could be increased, but low levels of inhibitors would still remain undetected since they cause minimal change in cell voltage.

A detailed step-by-step procedure for the preparation of enzyme pads for use in both the CAM-1 and CAM-4 electrochemical cells is given in Appendix F. Enzyme pads with activities in the range of 0.4 to 0.8 μmoles/min/pad are recommended since pads with higher activities are likely to be less responsive to the inhibitors, and pads with lower activities are likely to give intermediate baseline voltages (perhaps 300 mv) and are likely to fail sooner than the pads with more activity.

An applied current of 2 μA is chosen to yield a good spread of cell voltages between those encountered in the absence of enzyme inhibitors and those encountered in the presence of inhibitors. The use of higher cell currents could probably be tolerated if the concentration or flow rate of the substrate solution were increased.

To insure that sufficient pesticide contacts the enzyme immobilized on the pad and thus inactivates an appropriate fraction of the enzyme, the water sample is pumped through the pad at a rate of ∿ 200 ml/min. The pumping rate is not extremely critical, since--at this pumping rate--the amount of inhibition occurring in a single cycle is limited by the rate of diffusion of the inhibitor to the active sites of the gel-entrapped enzyme.

The following chemicals were purchased for use in the CAM-4 system:

- Butyryl cholinesterase Type IV-S from horse serum, Sigma Chemical Company, Product No. C-7512, approximately 15 units/mg (1 unit will hydrolyze butyryl choline to choline and butyrate at a rate of 1 μmole/min at pH 8.0 and 25°C.

- Butyrylthiocholine iodide, A Grade, Calbiochem Product No. 2049, melting point 172-173°C.

- Tris(hydroxymethyl)aminomethane, THAM, certified primary standard, Fischer Scientific Company, Product No. T-295. (Note: Several different lot numbers were submitted and we chose the best one on the basis of electrochemical cell performance, i.e., lowest in iron, copper, and other heavy metals.) Analytical grade hydrochloric acid from Mallinckrodt was used to adjust the pH of the 0.08 M solution in deionized water to prepare buffer. In previous studies, the substrate was found to be unstable because of a reaction occurring in the buffer. Although the chemistry of the reaction is unknown, undesirable changes can be avoided by treatment of the buffer with petroleum-based pelletized charcoal, MCB Company.

SECTION 7

OPERATING PROCEDURES FOR CAM-4

As explained in the previous sections, CAM-4 is not automatic but requires an operator in attendance all of the time when rapid detection of toxic materials is desired. Specifically, the operator must make up the substrate solution (one day's supply at a time), install the enzyme pads in the electrochemical cell when they are needed, determine when the enzyme pads are nearly exhausted so that he can replace them, and signal the presence of enzyme inhibitors when the baseline voltage increases 10 or more millivolts in one cycle. Most of these tasks are handled automatically in CAM-1, which is somewhat heavier, requires more power, and is more expensive than CAM-4.

When testing the CAM-4 instrument in the laboratory, it is necessary to connect the inlet hose to the water source and the outlet water hose to the drain; the unit is then connected to the appropriate power supply (either 12-v DC or 110-v AC). As a precaution, the water hose inlet for CAM-4 should not be connected directly to any pressurized water source since hoses may come off or the electrochemical cell may leak because of overpressurization. Water should be sampled from an overflowing beaker or similar container in a sink near the CAM-4, with the pump in CAM-4 used to suck the water into the system. The drain hose must be arranged so that the liquid drains freely and, accordingly, does not back up to fill the cell with liquid during the enzyme activity measurement part of the detection cycle. The simplified operating instructions given below will be of value to the reader or potential operator who is unfamiliar with the instrument.

STARTING PROCEDURE

1. Insure that the power is off.

2. Prepare fresh substrate solution by dissolving 40 mg of butyrylthiocholine iodide in 500 ml of 0.08 M Tris buffer, pH 7.4 (9.7 g of tris(hydroxymethyl)aminomethane in 992 ml of distilled or deionized water with enough concentrated HCl, $\sim$ 8 ml, to bring the pH to 7.4). Place the substrate solution in the plastic bottle in the lower left hand side of the instrument with the inlet end of the small plastic tube reaching the bottom of the bottle.

3. Open the electrochemical cell using the "cell open lever" shown in Figure 2 and remove the plastic enzyme pad holder. Place a fresh enzyme pad in the pad holder and insert into the electrochemical cell. Close the cell by pushing the "cell open lever" up.

4. Move the "AC or DC switch lever" up for AC or down for DC depending upon which power source is used.

5. Prime the substrate lines by moving the "substrate toggle switch" to the "up" position (red light comes on) for ∿ 3 to 5 min. When the line will not prime, replace the pump tube with a new silicone rubber pump tube (Scientific Industries, Tube No. SR-094).

6. Prime the sample water inlet line as follows:

   a. Place water inlet tube (Figure 2) into the water to be monitored. (If monitoring water in a lake or stream, place a screen or filter over the intake end of the tube. Open-pore urethane foam sponge makes a satisfactory filter.)

   b. Push "water toggle switch" to the "up" position (red indicator light should come on and the pump motor should start).

   c. Prime the pump by attaching a rubber vacuum/pressure bulb (similar to Fischer No. 14-087) to the drain line so as to suck water into the line and prime the pump.

7. Disconnect the rubber bulb and arrange the drain line so that water will drain out of it freely. (Caution: Back pressure on this line will cause water to remain in the cell during the voltage measuring part of the cycle and will make the system inoperative.)

8. After the water and substrate pumps are primed, place all toggle switches in the "down" or "automatic" position. The timing cycle will start and the indicator lights will come on in sequence to indicate the water sampling, the substrate pumping, and the applied current portions of the detection cycle are operating properly.

DISCUSSION OF CAM-4 OPERATION

Although the preceding steps put the instrument into operation, additional steps are necessary for the operator to interpret the numbers being generated by the digital printer. Specifically, the operator needs to know if his enzyme pad has any activity and how he can check the response of the instrument to specific solutions of enzyme inhibitors in order to calibrate its performance. Also, he needs to know when to replace the enzyme pad with a fresh one. The following paragraphs will help the operator understand what the instrument can do and what he must do.

Three definitions that will assist in the discussion to follow are these: starch pad potential, enzyme pad potential, and alarm potential. The starch pad potential is the voltage obtained when CAM-4 is operated with an exhausted enzyme pad or with a pad made like an enzyme pad but with no enzyme added to it. The voltage obtained with it is the highest voltage obtainable on the CAM-4 and is called starch pad potential or V_o (i.e., voltage with zero enzyme). Similarly, the enzyme pad voltage is the voltage obtained by CAM-4 when an enzyme pad is used in the electrochemical cell; it is designated as V_{enz} and varies with the amount of enzyme on the pad. The difference

between V_{enz} and V_o is called the alarm potential. By definition, the alarm potential is the change in voltage that would occur if all of the enzyme in an operating CAM-4 were inhibited. Normally, with good enzyme pads, the alarm potential varies from 200 to 300 mv. It is obvious that a spent enzyme pad cannot be used to detect enzyme inhibitors. To make certain that a working enzyme pad is always present in the electrochemical cell, the operator is instructed to replace the enzyme pad when the cell voltage comes within 50 mv of the V_o. For example, if an operator puts a starch pad in the electrochemical cell and determines that V_o is 350 mv, then he should make a record of this value and replace the working enzyme pad* before the cell voltage reaches 300 mv.

Because CAM-4 is not temperature-compensated and because there is often a difference in the dissolved solids in the water sampled, it is necessary to determine V_o at least once or possibly twice each day that the instrument is used.

Figure 11 shows typical printed cell voltages (in volts) obtained in the absence of inhibitors with the electrochemical cell operating on the 3-min cycle shown in Figure 4. During the water pumping part of the cycle (first 120 sec) the sampled water is pumped through the enzyme pad in the electrochemical cell at a rate of 200 ml/min. Some of the active sites on the enzyme pad will become inhibited by the inhibitors in the water, if they are present. For the last minute of the detection cycle, the water is turned off and air is blown through the cell. At the same time, the substrate flow starts. The rate of substrate hydrolysis is a function of the amount of enzyme activity left on the enzyme pad. A build-up of substrate solution in the cell is prevented by the stream of air. After the air and substrate pumps have been on for 20 sec, current is continuously applied to the electrodes in contact with the enzyme pad for 40 sec. Just before the current is turned off and the water pump is turned on again, the voltage at the electrodes is measured and printed on the paper tape. This voltage is low when the enzyme pad is active and increases when inhibitors are present. For the present study, we have assumed that a 10-mv increase or more in cell voltage in one 3-min detection cycle constitutes evidence for the presence of inhibitors in the water sample. This sudden increase in voltage is considered to be an "alarm" situation and is to be recognized by the operator since the instrument (unlike CAM-1) has no automatic method of flashing a light or sounding a horn.

Fresh enzyme pads will normally produce cell voltages, V_{enz}, that are at least 200 mv less than V_o. A new enzyme pad should keep the CAM-4 system in operation for a day or more, providing that inhibitors are absent from the water sampled; this is usually the case in a clean environment. However, the life of the pads is reduced in hot weather and they should also be protected from exposure to direct sunlight or humid environments. Thus, enzyme pads are normally stored in a dry, sealed container in the refrigerator.

* Caution: Always turn system off before opening cell to change pads.

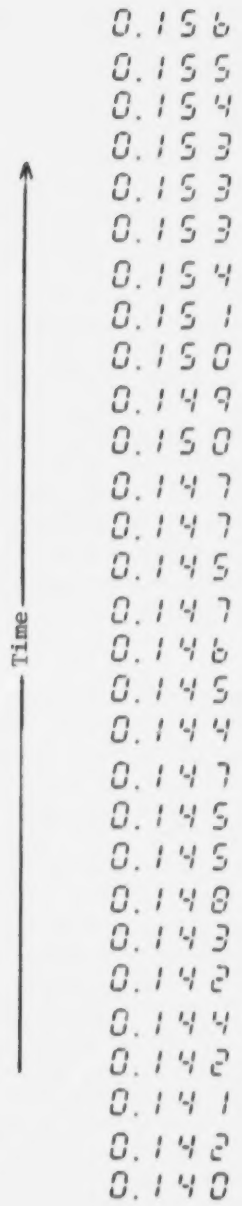


Figure 11. Digital printout in volts from CAM-4 showing cycle-to-cycle variation (noise) obtained when operating on a 3-min detection cycle in the absence of inhibitor.

MONITORING PROCEDURE

9. Follow the "Starting Procedure" (Steps 1-8) but insert a starch pad (no enzyme) into the electrochemical cell and sample uncontaminated or charcoal-filtered water.

10. Allow instrument to run for five 3-min cycles and record the last starch pad voltage, V_o.

11. Shut off CAM-4, insert fresh enzyme pad in cell, close cell, and turn on power.

12. Observe cell voltage, V_{enz}, for at least three cycles. Replace enzyme pad with fresh enzyme pad if V_{enz} is less than 150 mv lower than V_o.

13. Turn power off momentarily. Remove inlet hose from uncontaminated water and place in water to be monitored for inhibitors. Turn power on.

14. Observe paper tape to determine sudden increases of cell voltages, 10 mv or more per cycle, which indicate presence of inhibitors.

15. From time to time, check cell voltage, V_{enz}, to determine whether enzyme pad should be replaced, i.e., compare with V_o (Step 10 above) and replace if the difference is 50 mv or less.

16. Optional. To calibrate the system, prepare a gallon of water (3,785 ml) containing 0.2 ppm of DDVP (3.8 mg in 1 gal) at the same temperature as the water being sampled by the CAM-4. Switch from the uncontaminated water to the DDVP solution for a minimum of three water sampling cycles. The voltage response should average at least 10 mv/cycle. In the example, Figure 12, the average increase per cycle is 13 mv.

SHUT-DOWN PROCEDURE

17. Remove substrate and water inlet lines from their respective solutions and allow them to pump dry.

18. Remove the plastic substrate supply bottle from the case, discard the unused substrate solution, and rinse and dry the bottle.

19. Turn AC-DC power switch to "off."

20. Open electrochemical cell and remove enzyme pad holder; discard pad and wash pad holder.

21. For extended periods of disuse it is advisable to release the tension on the pumping tube of the substrate pump. This is easily achieved by lifting the tube off the rollers and pulling the tube toward the operator.

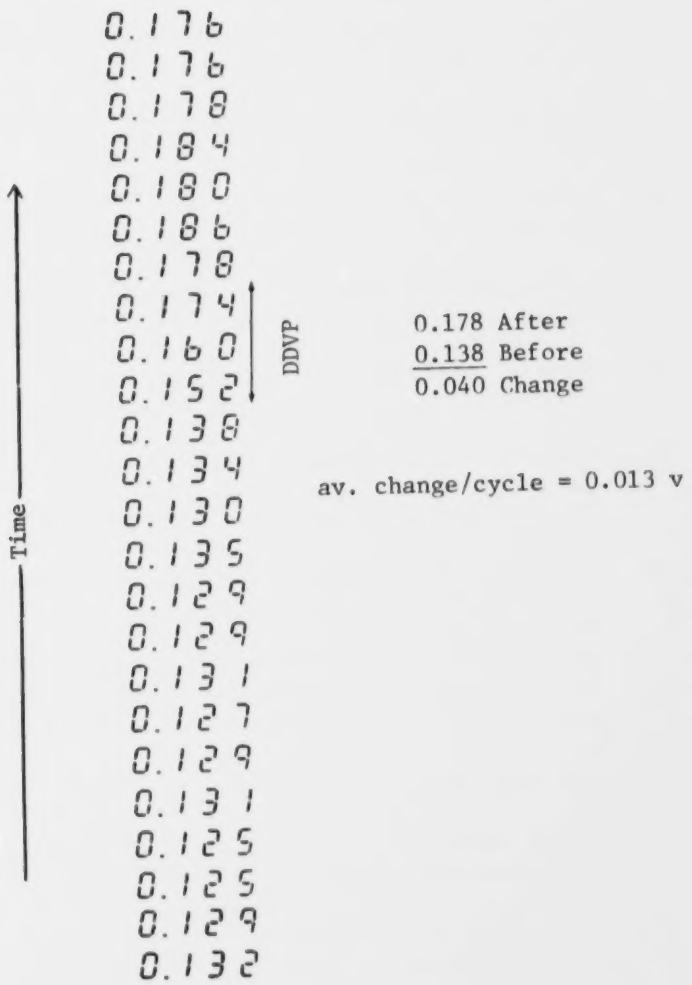


Figure 12. Typical calibration of CAM-4 showing cycle-to-cycle voltages before (lower portion of tape) and after (upper portion) exposure to 0.2 ppm DDVP for three cycles.

(Note: Since starch pad voltage for this run was 327 mv, the CAM-4 was able to continue monitoring with the same enzyme pad.)

SECTION 8

LABORATORY STUDIES WITH CAM-4

Testing of the two CAM-4 instruments built on this contract, namely, CAM-4-A and CAM-4-B, has centered around three specific goals: (a) response of CAM-4 to eight selected organophosphate and carbamate pesticides and subsequent determination of the sensitivity limit for each compound; (b) comparison of CAM-4 sensitivity to CAM-1 sensitivity for several representative pesticides; and (c) operation of CAM-4 under widely diverse field conditions for which CAM-4 was designed. All three of these goals were achieved during the laboratory and field tests of CAM-4 and are described in this and subsequent sections of this report.

The response of CAM-4 to eight commercial pesticides, presented in Tables 1 and 2, was determined in laboratory tests. Various quantities of each pesticide were dissolved in city tap water (15°C) and pumped through the instrument for five cycles. Voltage change was recorded for each of these cycles, plus one additional cycle; the responses were recorded for three trials. An average voltage increase of 10-mv or more per cycle (the total response divided by five) was considered sufficient for detection. The lower level of detection was considered to be that pesticide concentration that produced two or more 10-mv increases during the five cycle exposure period. Table 1 shows the response of CAM-4 (both systems A and B) to representative carbamate pesticides, while Table 2 shows the response of CAM-4 systems to representative organophosphate pesticides. In each of the tables, the lowest level of pesticide tested was considered to be the minimum detectable under the test conditions.

Sensitivities of the portable detection model, CAM-4, and of the laboratory model, CAM-1, were expected to be very nearly alike since the electrochemical and biochemical components of each are essentially the same, although the flow rates differ somewhat. Still, it seemed desirable to conduct a side-by-side comparison of response of the two systems to several pesticides. Table 3 presents the comparative study of the two systems to several carbamates and various organophosphates. As can be seen from the table, the total response in millivolts for each of the systems was very nearly the same during each of the three trials. One possible exception is the response of CAM-1 to Mesurol, which was slightly greater than any response on either CAM-4-A or CAM-4-B. No explanation for this difference is offered.

TABLE 1. RESPONSE OF CAM-4 TO CARBAMATE PESTICIDE SOLUTIONS[a]

System	Pesticide tested[b]	Level ppm[c]	Voltage response for each cycle, mv[d]						Total voltage change after five cycle exposure, mv[e]			Mean standard deviation	Alarm potential average[f]
			1	2	3	4	5	6	Trial 1	Trial 2	Trial 3		
CAM-4A	Mesurol[g]	12	11	13	15	14	18	0	71	57	66	65 ± 7.1	297
		8	7	13	14	11	10	-2	53	56	41	50 ± 7.9	290
CAM-4B	Mesurol	12	9	14	23	21	19	-1	85	83	87	85 ± 2.0	301
		8	12	8	15	16	15	2	68	73	72	71 ± 2.7	299
CAM-4A	Baygon[h]	20	7	18	13	18	13	2	71	101	78	83 ± 16.0	260
		15	8	18	9	13	10	6	64	54	53	57 ± 6.1	283
CAM-4B	Baygon	20	4	28	16	13	16	5	82	54	63	66 ± 14.0	280
		15	11	10	7	11	12	0	51	51	61	54 ± 5.8	260
CAM-4A	Sevin[i]	20	27	33	51	36	40	0	187	168	175	177 ± 9.6	255
		10	17	10	20	25	20	2	94	102	98	98 ± 4.0	280
		5	0	14	12	10	9	-3	42	45	43	43 ± 1.5	290
CAM-4B	Sevin	20	10	38	34	22	26	-2	128	136	115	126 ± 10.6	205
		10	20	20	17	23	13	10	103	86	90	93 ± 8.9	275
		5	0	7	10	9	8	3	37	28	31	32 ± 4.6	280

a Two CAM-4 units (A and B) were operated at 15°C using horse serum cholinesterase enzyme pads (4-5-76, 0.500 units activity). Substrate was 2.5×10^{-4} M butyrylthiocholine iodide in 0.08 M Fisher THAM, pH 7.4.

b A 1-liter stock solution of 1,000 ppm was made fresh for each pesticide. Insoluble pesticides were dissolved in a small amount of alcohol before mixing with 1 liter of pH 5.7 H_2O. The correct amount of stock solution was poured slowly into a stirred carboy of tap H_2O for sampling.

c Level tested is based on active ingredient.

d Exposure to stirred solution was for 5 cycles and voltage recorded for 6 cycles. Responses shown are for Trial 1.

e Three trials were run at each level for each system.

f Alarm potential is an average of all three trials and is calculated from the initial baseline using a fresh enzyme pad.

g Mesurol® = 4-(methylthio)-3,5-xylyl methylcarbamate.

h Baygon® = O-isopropoxyphenyl methylcarbamate.

i Sevin® = 1-naphthyl N-methylcarbamate.

TABLE 2. RESPONSE OF CAM-4 TO ORGANOPHOSPHATE PESTICIDE SOLUTIONS[a]

System	Pesticide Tested[b]	Level[c] ppm	Voltage Response[d] For Each Cycle, mv						Total Voltage Change After Five Cycle Exposure, mv[e]			Mean Standard Deviation	Alarm Potential Average[f]
			1	2	3	4	5	6	Trial 1	Trial 2	Trial 3		
CAM-4A	Nemacur[g]	0.4	17	21	23	19	19	-3	96	90	88	91 ± 4.2	284
		0.2	13	17	15	14	15	-2	72	62	68	67 ± 5.0	249
		0.15	7	10	9	13	10	-1	48	61	49	53 ± 7.2	285
		0.1	6	10	9	14	10	-1	48	39	44	44 ± 4.5	281
CAM-4B	Nemacur	0.4	19	35	40	32	5	-26	105	119	152	125 ±24.1	283
		0.2	16	30	24	25	2	-19	78	80	95	84 ± 9.3	280
		0.15	9	11	19	16	19	0	74	66	71	70 ± 4.0	307
		0.1	2	13	5	15	30	0	65	67	59	64 ± 4.2	251
CAM-4A	Baytex[h]	20	37	66	14	1	--	--	--	--	--	-- ± --	200
		10	29	22	20	10	10	-16	75	85	70	77 ± 7.6	252
		6	25	15	19	9	8	-10	66	60	63	63 ± 3.0	290
		5	24	5	5	4	11	0	49	39	42	43 ± 5.1	283
CAM-4B	Baytex	20	27	36	12	11	4	0	90	99	98	96 ± 4.9	262
		10	38	31	15	15	6	-30	75	72	57	68 ± 9.6	256
		6	23	15	12	11	17	-14	64	70	62	65 ± 4.2	250
		5	19	15	9	13	13	-22	47	54	43	48 ± 5.6	232
CAM-4A	Systox[j]	2	185	56	12	3	1	-9	249	257	229	245 ±14.4	273
		0.5	68	98	57	13	5	-17	224	229	215	223 ± 7.10	285
		0.1	27	29	22	25	18	-1	120	128	105	118 ±11.7	310
		0.05	10	9	17	12	5	21	74	72	78	75 ± 3.1	280
		0.025	10	11	23	3	12	-19	40	70	51	54 ±15.0	238
CAM-4B	Systox	2	109	48	25	22	3	-9	198	212	209	206 ± 7.37	266
		0.5	41	44	25	17	1	-8	120	132	141	131 ±10.5	273
		0.1	26	28	16	15	10	-2	93	100	98	97 ± 3.6	266
		0.05	6	14	8	11	6	6	51	63	67	60 ± 8.3	200
		0.025	10	11	3	11	7	6	48	57	46	50 ± 5.9	202

a Two CAM-4 units (A and B) were operated at 15°C using horse serum cholinesterase enzyme pads (4-5-76, 0.500 units activity)(responses to Systox used HSChE enzyme pads, 1-21-75, 0.513 units activity). Substrate was 2.5×10^{-4} M butyrylthiocholine iodide in 0.08 M Fisher THAM, pH 7.4.

b A 1-liter stock solution of 1,000 ppm was made fresh for each pesticide. Insoluble pesticides were dissolved in a small amount of alcohol before mixing with 1 liter of pH 5.7 H_2O. The correct amount of stock solution was poured slowly into a stirred carboy of tap H_2O for sampling.

c Level tested is based on active ingredient.

d Exposure to stirred solution was for 5 cycles and voltage recorded for 6 cycles. The responses shown are for Trial 1.

e Three trials were run at each level for each system.

f Alarm potential is an average of all three trials and is calculated from the initial baseline using a fresh enzyme pad.

g Nemacur® = ethyl 4-(methylthio)-M-tolyl isopropylphosphoramidate.

h Baytex® = 0,0-dimethyl O-(4-(methylthio)-M-tolyl)phosphorothioate.

i Electrical problems--system cycling erratically--did not finish response.

j Systox® = mixture (2:1) of 0,0-diethyl O-2-(ethylthio)ethyl phosphorothioate and 0,0-diethyl S-2-(ethylthio)ethyl phosphorothioate.

TABLE 3. COMPARATIVE RESPONSE OF CAM-4 AND CAM-1 TO PESTICIDE SOLUTIONS[a]

System	Pesticide tested[b]	Level ppm[c]	Voltage response for each cycle, mv[d]						Total voltage change after five cycle exposure, mv[e]			Mean standard deviation	Alarm potential average[f]
			1	2	3	4	5	6	Trial 1	Trial 2	Trial 3		
CAM-4A	DDVP[g]	0.3	10	10	11	7	9	-3	44	50	53	49 ± 4.6	240
CAM-4B	DDVP	0.3	9	11	7	12	14	-3	50	60	49	53 ± 6.1	235
CAM-1	DDVP	0.3	8	12	8	8	15	2	53	53	62	56 ± 5.2	286
CAM-4A	Furadan[Rh]	0.7	5	8	7	12	9	2	43	38	43	41 ± 2.9	257
CAM-4B	Furadan	0.7	10	7	13	8	8	-1	45	40	37	41 ± 4.0	223
CAM-1	Furadan	0.7	5	18	10	9	3	5	50	39	44	44 ± 5.5	260
CAM-4A	Systox[Ri]	0.15	19	12	4	13	15	10	73	47	60	60 ± 13	267
CAM-4B	Systox	0.15	21	9	13	26	12	2	83	50	64	66 ± 17	250
CAM-1	Systox	0.15	20	18	22	5	17	-10	72	49	75	65 ± 14	250
CAM-4A	Mesurol[Rj]	8	8	14	12	12	12	8	66	51	50	56 ± 9.0	245
CAM-4B	Mesurol	8	11	11	15	8	17	-4	58	40	63	54 ± 12	255
CAM-1	Mesurol	8	12	19	16	16	19	-6	76	60	68	68 ± 8.0	275

a Two CAM-4 units (A and B) and one CAM-1 unit were operated at 10°C using horse serum cholinesterase enzyme pads (5-24-76, 0.326 units activity). Substrate was 2.5×10^{-4} M butyrylthiocholine iodide in 0.08 M Fisher THAM, pH 7.4 (Tris buffer).

b A 1-liter stock solution of 1,000 ppm was made fresh for each pesticide. Insoluble pesticides were dissolved in a small amount of alcohol before mixing with 1 liter of pH 5.7 H_2O. The correct amount of stock solution was poured slowly into a stirred carboy of tap H_2O for sampling.

c Level tested is based on active ingredient.

d Exposure to stirred solution was for 5 cycles and voltage change recorded for 6 cycles. The responses shown are for Trial 1.

e Three trials were run at each level for each system.

f Alarm potential is an average of all three trials and is calculated from the initial baseline using a fresh enzyme pad.

g DDVP = 0,0-dimethyl 0-2,2-dichlorovinyl phosphate.

h Furadan[R] = 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate.

i Systox[R] = mixture (2:1) of 0,0-diethyl 0-2-(ethylthio)-ethyl phosphorothioate and 0,0-diethyl S-2-(ethylthio)ethyl phosphorothioate.

j Mesurol[R] = 4-(methylthio)-3,5-xylyl methylcarbamate.

SECTION 9

FIELD TESTING OF CAM-4

Both CAM-4 instruments were operated under a wide variety of field conditions during their evaluation. The purpose of the field tests was two-fold: (a) to gain experience in transportation of the system and the auxiliary equipment required to operate and maintain the unit, including the 12-v power source, filter sponge, tubing, solutions, pH paper, thermometer, tools, pads, etc.; and (b) to monitor the performance of the CAM-4's under natural environmental conditions. Several parameters were recorded at each location during the field operations. These included water temperature and pH, air temperature, condition of the sample water (clean, turbid, debris, flowing or stagnant), and any other environmental conditions specific to each site.

Observations were made with respect to false alarms, baseline stability, and alarm potentials at each location. A log was kept during the monitoring of each location and observations were entered approximately every hour. Problems encountered during each field test were recorded so that these could be evaluated and corrected before initiation of subsequent tests. Part of the test protocol at each sample site was an exposure to a standard solution of DDVP (2,2-dichlorovinyl dimethyl phosphate) made up in a large container with the water at the site. This system check was done to ascertain the sensitivity of the system with the raw water actually being sampled. A brief description of five field tests with CAM-4 is given in Appendix C of this report.

Samples obtained from the Kansas City Municipal Water Treatment Plant, the Missouri River (raw, untreated), several areas of a farm pond, a storm drainage ditch, and the effluent from a local pesticide manufacturing plant were tested with the CAM-4. No significant difficulties were encountered. When CAM-4 is used under field conditions, such as those described below, it is essential to attach a filter to the water inlet line of CAM-4 to remove debris that might cause plugging of the platinum electrodes or of the one-way valve in the sample line. With the sample line inserted into the center of a small cube of urethane foam (5 in. on an edge), all field tests with the instrument proved to be satisfactory. The urethane foam served both as a filter and as a float to keep the inlet line just below the water surface, thereby preventing air bubbles from entering the water pump. When CAM-4 is operated from a small boat, it is essential that no part of the drain tube from the cell be higher than the bottom of the cell, i.e., water must not be allowed to collect in the drain tube since this produces back pressure on the electrochemical cell and results in an erratic baseline. Good drainage is easily achieved by setting the instrument on the boat seat rather than on the floor of the boat.

On the basis of the field tests, it is concluded that the CAM-4 instruments performed well under a wide variety of environmental conditions and were able to detect the presence of toxic levels of cholinesterase inhibitors in water downstream from the discharge of a pesticide manufacturing plant. The systems would be easier to operate if self-priming water pumps were used to replace the present gear pumps, but such pumps in the appropriate size and weight do not seem to be available commercially at this time. (See Appendix C for Field Test with CAM-4.)

SUMMARY OF CAM-4 OPERATION IN THE FIELD

1. Both CAM-4's were operated in the field in an air temperature range of $6^{o}C$ to $21^{o}C$.

2. Water temperature tested at the five locations ranged from $8^{o}C$ to $23^{o}C$ while pH varied from 5 to 7.5.

3. CAM-4 was operated for a total of 31-1/2 hr in the field.

4. During the field tests, a floating urethane sponge filter was used on the water inlet line to prevent plugging and proved satisfactory under widely varying conditions.

5. No false alarms occurred during the 31-1/2 hr of testing although a minor electrical problem was encountered with one printer.

6. No electrical or chemical interference problems were encountered during the field tests.

7. At each location during field operation, CAM-4 was exposed to a standard sample of DDVP. A rapid increase in the voltage demonstrated that the system was functioning properly.

8. CAM-4 proved to be portable, was easily operated from a river bank or from a small boat, and performed well in clear, drizzly, or humid weather.

9. A fully charged 12-v automobile battery provided sufficient DC power to operate the CAM-4 continuously for 8 hr.

10. No problems were encountered in pad changing and the substrate solution was easily prepared in the field.

APPENDIX A

ABSTRACT OF CONTRACT ON CAM-1 DEVELOPMENT

Rapid Detection System for Organophosphates and
Carbamate Insecticides in Water

Environmental Protection Technology Series EPA-R2-72-010, August 1972

(Final Report, Contract No. 68-01-0038)

An apparatus for the detection and monitoring of water supplies for hazardous spills of organophosphate and carbamate insecticides has now been designed and fabricated. The new unit is called the Cholinesterase Antagonist Monitor, CAM-1, because it produces an alarm in 3 min when toxic or subtoxic levels of cholinesterase antagonists are present in water. Response of this apparatus to subtoxic levels of azodrin, Sevin®, dimetilan, malathion, parathion, and DDVP has already been demonstrated. CAM-1 uses immobilized cholinesterase for the collection of cholinesterase inhibitors from the water supplies. The activity of the immobilized cholinesterase is determined automatically in an electrochemical cell by passing a substrate solution over the enzyme at regular time periods. A minicomputer is used to automate the detection process and to signal an alarm when there is a rapid loss of enzyme activity--a situation that occurs in the presence of organophosphate and carbamate insecticides in the water sampled.

This report was submitted in fulfillment of Project No. 15090-GLU, Contract No. 68-01-0038, under sponsorship of the Water Quality Office, Environmental Protection Agency.

APPENDIX B

ABSTRACT OF TASK I REPORT ON THIS CONTRACT

Evaluation of "CAM-1," A Warning Device for Organophosphate Hazardous Material Spills

Environmental Protection Technology Series EPA-600/77-219, November 1977

(Final Report on Task I, Contract No. 68-03-0299)

The Cholinesterase Antagonist Monitor (CAM-1) has been operated with water containing a variety of pollutants including organophosphates, carbamates, chlorinated hydrocarbons, and various other economic poisons, and its sensitivity to many of these materials has been measured. With few exceptions, only the organophosphates and carbamates are detectable with CAM-1. One of these exceptions is zinc, at 10 ppm, which inactivates cholinesterase and behaves in CAM-1 like the organophosphates. Another compound detectable under certain conditions is the reversible cholinesterase antagonist, tributyl amine hydrochloride; it is detectable for only one or possibly two cycles when a sudden increase in the concentration of the reversible inhibitor occurs. The non-reversible enzyme inhibitors, on the other hand, produce repeated voltage increases until the enzyme in CAM-1 is completely inactivated. CAM-1 is recommended only for the detection of non-reversible inhibitors.

Correlation of the sensitivity of CAM-1 with the chemical structures of a group of organophosphate pesticides has shown that CAM-1 is generally more sensitive for the phosphate (-O-P(=O)-) compounds than for the phosphorothioate (-O-P(=S)-) or the phosphorodithioate (-S-P(=S)-) compounds, even though the animal toxicities of these different types of compounds may be very close.

Operation of CAM-1 in simulated sea water (3% NaCl) changes the voltages registered on the digital voltmeter, but it does not change the sensitivity of CAM-1 for compounds like DDVP; thus, CAM-1 is suitable for the detection of cholinesterase inhibitors in either sea or brackish waters. CAM-1 has much promise for monitoring of water supplies and plant effluents for cholinesterase inhibitors, but it is so new that it should be investigated under the conditions of intended usage prior to putting it into regular service.

APPENDIX C

CAM-4 FIELD TEST REPORT

The first site for field testing was a 7-acre lake located on an experimental farm in Grandview, Missouri. The lake is spring fed, contains fish and weeds, and remains completely natural except for a large aerator located at its center. In this test, CAM-4 was operated in a row boat from a 12-v automobile battery at various points in the lake. The water temperature ranged from 19°C at the center of the lake to 23°C in the shallow areas at the edge. The pH of the water, determined with test paper, was 6.0.

For the initial testing, a thin open-pore polyurethane foam filter was placed on the water inlet line to keep out lake scum and debris. The filtering capacity of the foam was satisfactory, but the filter did not float and had to be attached to the boat. Problems were encountered with this method of filtering. The filter had a tendency to drag along the bottom and fill with mud in the very shallow areas. The problem was corrected by raising the line closer to the water surface; however, excess movement of the boat allowed air to enter the line and stop the water flow. The operator was required to re-prime the water pump. Floating inlet filters, as described earlier in Section 8, were used for subsequent testing.

Baselines were smooth throughout the 6-hr test period, no inhibitor was detected, and no false alarms occurred. An exposure to 0.3 ppm DDVP in lake water gave a total change of 60 mv in five cycles showing that the system was performing in the same manner that it had performed in the laboratory.

One minor problem was encountered in operating the portable detector from a row boat; the drain tube retained water during the substrate pumping part of the cycle and produced an erratic baseline. The problem was solved by operating the detector from the boat seat rather than from the floor of the boat, such that the drain tube was lower than the electrochemical cell.

The second test site was in Brush Creek which runs through the business and residential area of Kansas City, Missouri. This creek collects much of the storm drain runoff of the area and may even contain sewage at times. CAM-4 was operated on a 12-v automobile battery from the creek bank and remained at one location throughout the 7-1/2 hr test period.

At this site, the initial water temperature was 6°C with a pH of 5, as measured by test paper, and air temperature was 9°C. During the test period, the water temperature increased to 10°C and air temperature to 19°C. This temperature change had no effect on the baseline stability and no false alarms occurred. The response of CAM-4 to 0.3 ppm DDVP mixed with the creek water was slightly lower than that obtained in the laboratory (52 mv), but

the responses signalled in two cycles, 4 mv and 10 mv, were enough to consider the instrument to be operational. No inhibitors were detected in the creek at this location.

A new water inlet line filter was tried at the Brush Creek site. The inlet filter was a 5-in. square cube of urethane foam sponge with a hole drilled in the center into which the inlet tube was inserted. This cubic sponge floated just below the surface and proved to be very satisfactory, both as a filter and a float.

The water at this creek site was running rapidly and was very clear. To test the filtering efficiency of the cube sponge, large clouds of dirt, silt, leaves, and other debris were stirred up by agitating the creek bottom 20 yards (18 m) upstream from the CAM-4 and allowed to travel downstream past the instrument. The filter became dark brown during the 7-1/2 hr run, but no visible material entered the water line, and the baseline was unaffected.

A minor electrical problem was encountered with the digital printer at this location. Spurious extra digits, other than those showing the cell voltage, were printed in columns. This did not seem to interfere with the correct voltage reading and when CAM-4 was returned to the laboratory for examination, the fault was traced to a bad connection.

The next field location to which the CAM-4 was taken was again the experimental farm lake in Grandview, Missouri. The weather was much cooler than during the previous tests here, allowing an assessment of the effect of temperature on CAM-4. The new floating filter could be evaluated while moving in a boat along the edges and shallow areas of the lake where there was much debris. These areas had been inaccessible to monitoring using the previous non-floating water inlet-line filter.

The CAM-4 was again operated from a 12-v automobile battery and was moved to various points around the lake in a small row boat. The water temperature here was 10°C, with a pH of 6 (test paper) at all locations. Air temperature was 10°C throughout the 7-hr test period; the air was very humid, and there was a slight drizzle for about 1 hr during the testing.

The CAM-4 monitor was next taken to the shallow end of the lake where much floating debris (leaves, bark, duckweed) and large amounts of bottom algae were present. The filter was tested in a stationary position among this debris for 1 hr. No effect was seen on the baseline during this period, but the filter became covered with scum after 1 hr and a slight reduction in water flow rate was noticed (about 50 ml/min). Removal of the filter and a quick rinsing in lake water alleviated this problem. The filter was also tested at the spillway of the lake where there was a large accumulation of a very fine green silt-like material, both floating and suspended in the water. This material passed through the filter, as evidenced by the greenishness in the tubing, and resulted in a slightly more erratic baseline though no false alarms occurred. Excessive foaming was noticed at the drain tube as this material passed through the system. The

filter floated satisfactorily during the test period independently of whether the boat was moving or stationary.

In the field, the standard exposure test to 0.3 ppm DDVP was performed. Total response was 40 mv, while one 10-mv "alarm" situation occurred. This response was less than that obtained during laboratory testing and may possibly be attributed to the colder testing conditions. Although CAM-4 becomes less sensitive as the temperature decreases, it retains sufficient sensitivity to detect subtoxic levels of all those compounds tested. Without changing the enzyme pad, its use was continued for several cycles and then exposure to 5 ppm DDVP was tested. The result was a 136-mv increase in three cycles and complete inhibition of the enzyme pad.

This 136-mv voltage increase demonstrated in the field that a partially inhibited enzyme pad still has the ability to respond well when high levels of inhibitor are encountered.

CAM-4 was taken to another small stream as its fourth field test site. This stream (Rock Creek) runs through the back property of residences in suburban Mission, Kansas, and is actually little more than a drainage ditch. At this location, the water temperature was 8°C initially and rose to 10°C during the 5-1/2 hr test period (pH of 7.5). Air temperature was 6°C and rose to 10°C during the testing. The stream contained dead twigs and tree branches. There were numerous cardboard containers, beer cans, and bottles along the bank and in the stream. The water was moving very slowly and was almost completely covered with floating leaves (birch, cherry, black oak, weeping willow, and apple). The stream passed through a landfill/garbage dump 50 yds upstream from where the CAM-4 was located, and also caught runoff water from residences in the immediate area.

The standard exposure test to 0.3 ppm DDVP was done at this location, as on all the other field tests. Response was a total change of 68 mv with three alarms of 10 mv or more--slightly larger than expected for such cold weather conditions.

No significant problems were encountered during the entire 6 hr of testing at the Rock Creek location. No inhibitor was detected, the baseline was very stable, and no false alarms occurred. Plugging of the water line filter, which was not unexpected since the majority of the debris being filtered was very large, was not observed. There were no electrical or mechanical failures. All in all, this was a very satisfactory field test.

CAM-4 was taken to one final field location for testing. The instrument was used to monitor the effluent of a local pesticide manufacturing plant where process wastewater poured into a major tributary of the Missouri River (Little Blue River) in Kansas City, Missouri. The manufacturing plant is located in an industrial district traversed by the stream. Upstream from the pesticide manufacturer's facility are several other industrial establishments including a grain elevator and feed packing plant, a chemical company, a steel works, an electric generating station, and a sewage treatment plant.

CAM-4 was operated from the river bank using a 12-v automobile battery during the 6-hr test period. Two separate areas were monitored at this site: the point at which the effluent entered the stream, and a point approximately 15 yards (14 m) upstream. Access to these locations was made by foraging through thick brush and some dense undergrowth along the upper bank. The effluent itself was pink/red and foaming, and had a strong unidentifiable chemical smell. The dark brown muddy stream flowed slowly but was clear of floating debris. Visible amounts of a black oily sludge-like material had accumulated along the edge of the bank, on rocks, and on tree stumps up and down the stream from the monitoring location. The water temperature was 12°C with a pH of 6. The pesticide plant effluent pH was 4 to 5 as determined by test paper. Air temperature was initially 11°C and rose to 21°C during the period of testing.

No mechanical or electrical problems were encountered during this test, but high concentrations of enzyme inhibitor were present at the effluent monitoring site. Three enzyme pads were used and completely inhibited during the first 1-1/2 hr of operation. The alarm potential was slightly low at the start of testing at the effluent site (160 mv versus 240 mv in the lab) but this was attributed to the partial inhibition of the enzyme pad due to the contaminated water used to determine the baseline voltage (V_{enz}). Monitoring of the effluent as it moved downstream would have been interesting, but no boat was available. Relocation of the detector downstream from the effluent gates was also impossible because the river bank was inaccessible for several miles.

The portable detection apparatus was moved 15 yards (14 m) upstream from the first location and monitoring was again initiated. The alarm potential at this location was more acceptable (225 mv) and much less inhibitor was detected as compared to direct sampling of the effluent. Since the enzyme pad baseline continually rose at about 5 mv/cycle, it was evident that low levels of inhibitor were present but no alarms occurred. Moving the detector and storage battery from one stationary location to another along the overgrown bank was difficult and time consuming for one operator. In the future, when it is desired to sample several locations in a short period of time, it would be more efficient to operate the detector from a small boat.

APPENDIX D

CAM-4 PARTS LIST *

Parts	Description	Manufacturer or Supplier
DVM BOARD (FIGURE 5a)		
Printed Circuit Board		Teletron
ADC-1100	Analog to Digital Converter	Analog Devices
IC1	LM741CN Operational Amplifier	National Semiconductor
IC2	7400 Quad Name Gate	National Semiconductor
IC3	MC846P	Motorola
T1	2N3904 NPN	Semiconductor Specialists
T2	2N3905 PNP	Semiconductor Specialists
T3	2N3565 NPN	Semiconductor Specialists
T4	MPF102 FET	Semiconductor Specialists
Z1	ZB82A Zener	Semiconductor Specialists
D1, D2	1N914 Diodes	Semiconductor Specialists
C1, C2	22 µf/25 v Tantalum caps.	Newark
C3	0.02 µf Mylar cap.	Newark
R1, R5	3.3 KΩ 1/4 w Resistor	Newark
R2	1.5 KΩ 1/4 w Resistor	Newark
R3	1.0 KΩ 1/4 w Resistor	Newark
R4	2.7 MΩ 1/4 w Resistor	Newark
R6	470 Ω 1/4 w Resistor	Newark
P1	50 KΩ Trimpot 3006P-1-503	Bourne Newark
P2	1 MΩ Trimpot 3006P-1-105	Bourne Newark
* Resistors are 1/4 w unless otherwise identified.		
POWER SUPPLY BOARD (FIGURE 5b)		
Printed Circuit Board		Teletron
BR1	Bridge Rectifier W110	Semiconductor Specialists
BR2, BR3	Bridge Rectifier KBPC8005, KBPC1005	Semiconductor Specialists
Regulator 1	7805	Semiconductor Specialists
Regulator 2	4195	Semiconductor Specialists
IC1	LM555CN	National Service
C1, C2	220 µf/35 v Electrolytic	
C3	5,000 µf/10 v Electrolytic	
C4	0.1 µf Electrolytic	
R1	3.3 Ω, 1/2 w	
R2	220 Ω 1/4 w	
TIMER AND TRIAC SWITCH BOARD (FIGURE 7)		
Printed Circuit Board		Teletron
IC1	Timer LM555CN	National Semiconductor
IC2, 1C3, 1C4	TTL Decade Counter 7490	National Semiconductor
IC5	Decade Decoder 7442	National Semiconductor
IC6	Triple 3 input Nand 7410	National Semiconductor
IC7	Dual 4 input Nand 7420	National Semiconductor
IC8	8 input Nand 7430	National Semiconductor
IC9, IC10	Optical Isolators 7N28	Motorola
IC11	DTL Gates 660P	Motorola
T1	2N3905	Semiconductor Specialists
T2, T3, T4, T5	2N3565	Semiconductor Specialists
T6	2N3906	Semiconductor Specialists
T7, T8	RCA Triac Type 40526	Semiconductor Specialists
D1, D2	Trigger Diode MB54991	Semiconductor Specialists
D3	IR170 Rectifier	Semiconductor Specialists

Parts	Description	Manufacturer or Supplier
TIMER AND TRIAC SWITCH BOARD (FIGURE 7) Contd.		
Z1	Zener (15 v) ZB15A	Semiconductor Specialists
P1	1 MΩ Trimpot Spectral Type 43P105	Semiconductor Specialists
P2	50 KΩ Trimpot Spectral Type 43P504	Semiconductor Specialists
C1	1 µf Mylar	Newark
C2, C3, C6	0.005 µf	Newark
C4	4 µf/250 v Electrolytic	Newark
C5	0.022 µf Electrolytic	Newark
R1, R2, R3, R4	1.2 K Electrolytic	Newark
R5	10 K Electrolytic	Newark
R6	1,500 Ω Electrolytic	Newark
R7, R8, R9, R10	3.3 K Electrolytic	Newark
R11, R12, R13, R14	180 Ω Electrolytic	Newark
R15, R16	42 K Electrolytic	Newark
R17, R18	8.2 K Electrolytic	Newark
R19	3 KΩ 6 w Electrolytic	Newark
INVERTER AND OTHER PARTS (FIGURES 2, 3, 6, 8)		
Case	Model 92500	Skydyne, Inc.
Hardware Mounting Panels		Teletron
Printer	DPP-7	Datel
Inverter	Model 12-115	Nucleonic Products, Inc.
Transformer	UP6377	
Transformer	40 v CT Type 18A1487	Burstein Applebee
Regulator (5 v)	LM309	
PC Sockets	225-22221-401(117)	Amphenol
Card Guide (6 req.)	T-309-48	Cambion
Spacers	T-101-300	Cambion
Extractor (6 req.)	S-200	Cambion
Extrusions for card cage	XTS-802-36	Cambion
Binding Posts	Type 29-1	Grayhill
SW1	Type 7693K2 4PDT	Cutler Hammer
SW2, SW3, SW4	MTA106D	Alco
Leds (3 req.)		
AC Connector		
AC Power Cord		
Water Pump, Gear	Delrin Plastic No. 7012	Cole Parmer
Air Blower	Sprite Tubeaxia/fan	Rotron
Substrate Pump	Rotor	Scientific Industries
Substrate Pump	Tubing Support	Scientific Industries
Substrate Pump	Outboard Bearing Plate	Scientific Industries
Air Pump	Aquarium Type	Hush
Electrochemical Cell Holder		Teletron
Electrochemical Cell Injection Molded with Platinum Anode and Cathode		MRI

APPENDIX E

MECHANISM OF ELECTROCHEMICAL DETECTION PROCESS IN CAM-4

OXIDATION POTENTIAL THEORY

This theory proposes that cholinesterase is able to convert butyrylthiocholine iodide (BuSChI) into butyric acid and thiocholine iodide (HSChI). Since the thiol is easily oxidized, a low voltage is measured by the constant current electrochemical system. In the absence of enzyme, there is no thiol present and the voltage rises--usually about 250 mv since the original substrate, BuSChI, is not readily oxidized. It has been proposed that the voltage decrease is due to the oxidation of I^- to I_2. This explanation has appeal, but it does not explain (a) how this iodide-to-iodine oxidation potential can vary from 100 mv to 600 mv as the electrodes are conditioned; (b) why no trace of iodine color has ever been detected on the starch covered enzyme pad; and (c) why the voltage does not immediately fall to zero since traces of iodine completely depolarize the electrodes.

Supporting this theory is the knowledge that HSChI is readily oxidizable to the disulfide and it can be found among the products coming through the electrochemical cell. If this theory is correct, the voltage change should be observable with a number of electrode pairs at equivalent solution concentrations. We have not found alternate electrode materials that give as large voltage changes as platinum.

ANODE DEPOLARIZATION THEORY

This theory suggests that the anode is coated with a layer of platinum oxides or sulfides, and perhaps other materials, tend to reduce its electrical conductivity. Exposure of this coated anode to a solution containing a trace of thiol results in a controlled depolarization, or increase in conductivity of the anode coating. In favor of this theory is the finding that application of a direct current to two identical electrodes for a few minutes in the presence of hydrolyzed substrate (i.e., HSChI) produces two stable, but dissimilar electrodes that generate voltage like a battery when they are placed in an electrolyte. The electrodes are readily made alike, or depolarized, by treatment with a trace of free iodine or chlorine; such treatment in our electrochemical system drives the voltage to zero, indicating excellent conductivity. The electrodes recover after the halogen is gone.

In beaker experiments, a standard calomel electrode is used as a reference electrode while current is applied to two identical platinum electrodes in a solution; when the solution is changed from HSChI to BuSChI, it is noted

that nearly all of the voltage change occurs at the anode. This suggests that the conductivity of the anode surface is changing with the change of material in the beaker. Measurement of applied current in the electrochemical cell with an enzyme pad showed that it was sufficient to oxidize only about 5% of the HSChI produced by the enzyme pad. Presumably a close balance between coulombs of applied current and moles of HSChI would be required to obtain rapid response of the system to enzyme inhibitors.

The disulfide of thiocholine iodide found in the products coming from the cell could arise either from air or electrochemical oxidation of the HSChI. No evidence has yet been gained for either theory. With freshly-plated platinum electrodes, the voltages obtained with enzyme pads are often as low as 0 mv at first, and after the electrodes have been used for a while (e.g., a day or two), the enzyme pad voltage may be as high as 250 to 300 mv; at the same time, the voltage change obtained when replacing an enzyme pad with a starch pad (no enzyme) is 200 mv or more whether the electrodes are new or conditioned. This suggests that the enzyme pad voltages obtained are not characteristic of the oxidation potential or thiocholine iodide since they range from 0 to 300 mv.

In summary, the exact mechanism of the electrochemical reaction is unknown; both electrode polarization and thiocholine iodide oxidation may be occurring simultaneously in the electrochemical cell. The mechanisms enabling electrochemical estimation of enzyme pad activity are worthy of further investigation.

Further support to the anode depolarization theory is given by Kramer, et al.,(1) who reported constant current experiments in which depolarization of a platinum anode by thiocholine iodide resulted in increased conductivity of the anode. This electrochemical reaction forms the basis of their procedure for analysis of cholinesterase and thiocholine esters.

(1) Kramer, D.N., P.L. Cannon, Jr., and G.G. Guilbaut. Electrochemical Determination of Cholinesterase and Thiocholine Esters. Anal. Chem., 34(7):842-845, 1962.

APPENDIX F

ENZYME PAD PREPARATION PROCEDURE

Care must be taken in the preparation of the enzyme pad for use in CAM-4 since the sensitivity of CAM-4 and the repeatability of the tests are a function of the uniformity of the enzyme pads. Timing and manual dexterity are important procedural factors in the preparation of the pads. It is suggested that the individual selected to prepare the pads should practice the handling of the starch and its application to urethane foam as mentioned below, but with a water-soluble dye substituted for the enzyme. In this way, it will be possible to anticipate changes in starch viscosity and to check out the procedure for uniform distribution of the starch applied to the foam. After gelling and drying, the starch-coated foam should then be cut into pads, since starch pads are also needed for the determination of CAM-4's alarm potential at the beginning and ending of each day's tests.

(The alarm potential is the voltage increase which occurs when all of the enzyme on the pad is inhibited or when the enzyme pad is replaced with a "starch pad" to which no enzyme has been applied. Daily checks of the alarm potential are suggested as a means to prove that the instrument is working.)

The following materials were used: open-pore polyurethane foam sheets, 44 to 45 pores per linear inch (ppi) x 1/4 in., Scott Industrial Foam, Scott Paper Company, Chester, Pennsylvania (distributed by Crofton, Inc., 1801 West Fourth Street, Marion, Indiana 46952); partially hydrolyzed potato starch recommended for gel electrophoresis, Connaught Medical Research Laboratory, Toronto, Canada; Chlorhydrol^{R} (aluminum chlorhydroxide complex, 50% w/w solution), Reheis Chemical Company, Chicago, Illinois; horse serum cholinesterase, Sigma Chemical Company, Type IV, approximately 15 M units/mg; and Tris buffer, "THAM"^{R} Fischer Scientific.

Step 1 A solution of Tris buffer, 0.08 M, was prepared by dissolution of 9.7 g of tris(hydroxymethyl)amino methane in 900 ml of water, adjustment of the pH to 7.4 with concentrated HCl, and then adjustment of the volume to 1 liter.

Step 2 One hundred twenty-five milligrams of horse serum cholinesterase were dissolved in 6 ml of Tris buffer. To this solution were added, with mechanical stirring, 4 ml of a dilute solution of aluminum chlorhydroxide complex (0.03 g of Chlorhydrol^{R} in 4 ml of water). At this point the aluminum hydroxide gel precipitated and adsorbed the enzyme from the solution (adjusted pH to 7.4-8.0 to ensure that the precipitation was complete). This suspension was set aside at ambient temperature until needed in Step 3.

Step 3 Two grams of potato starch were suspended in 10 ml of cool Tris buffer, added to 30 ml of boiling Tris buffer, and heated until the suspension cleared. Care was taken to avoid the formation of scum or lumps (start Step 3 over if lumps are obtained) and the starch slurry was stirred with a magnetic stirrer while it cooled slowly to 45°C. At this point, the aluminum hydroxide gel-entrapped cholinesterase (Step 2) was added all at once and quickly mixed. (Note: Step 4 and its three replications must be done quickly before the starch gels.)

Step 4 A 10-ml portion of the warm starch gel slurry from Step 3 was deposited on a pre-cut sheet of open-pore urethane foam (4 x 6 x 1/4 in. sheet) lying on a warmed glass or plastic surface (usually over a pan of warm water). The starch-enzyme material was now distributed throughout the sheet as uniformly as possible with a plastic rolling pin filled with warm water; the sheet was rolled in all directions and turned over several times. In this same manner, three other 10-ml portions of the starch-enzyme product were distributed over three additional urethane foam sheets.

Step 5 The coated sheets were placed on edge in a wooden rack (made with dowel rods), dried at least an hour at room temperature, and finally dried overnight in an oven at 37°C.

Step 6 The resulting sheets were examined carefully to ensure that all areas of all pads were evenly coated. Poorly-coated areas were trimmed away. The sheets were handled carefully to avoid breakage of the starch film on the dried sheets. The enzyme pads were next cut into 3/8-in. diameter pads with a stainless steel cutter mounted in an electric drill press. The procedure yielded approximately 350 enzyme pads, which possessed an average activity of 0.5 units/pad (analysis based on the rate of hydrolysis of butyrylthiocholine iodide and measured by a modification of the Ellman Procedure).[1]

Step 7 The enzyme pads were then placed in a screw-capped bottle and stored in a second container, with dessicant, in a refrigerator until needed. Pads made and stored in this manner retain their usefulness for more than a year.

(1) Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochem. Pharmacol., 7, 88-95, 1961.